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CHEMICAL AND PHYSICAL INVESTIGATIONS

INTRODUCTION

BY

E. F. THOMPSON, M.Sc.(N.Z.), PH.D.(CANTAB.) AND
H. CARY GILSON, M.A.

WITH SIXTEEN TEXT-FIGURES AND ONE CHART

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INTRODUCTION

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E. F. THOMPSON, M.Sc.(N.Z.), Ph.D.(Cantab.) and
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WITH 16 TEXT-FIGURES AND ONE CHART.

The results recorded in the present volume are those of the hydrographical and chemical investigations carried out by the John Murray Expedition of September, 1933, to May, 1934. A general account of the ship and apparatus and a brief narrative of the cruise are given in the previous volume,* where a map showing the course sailed and the positions of stations is attached. The present introduction is confined to the special methods and conditions affecting the hydrographic results. Two sections of the chemical work are dealt with separately by other authors. Mr. Gilson deals with the nitrogen cycle in the area, with particular reference to its interrelations with plankton production; while Mr. Mohamed considers the values of hydrogen-ion concentration. The details of the methods employed by these two workers will be given in their particular sections.

The aims of the hydrographic work were twofold. First, the characterization of the different water masses, their circulation, and the effects of the seasonal winds on this circulation; secondly, the correlation between these facts and the biological conditions.

APPARATUS AND EQUIPMENT.

(*a*) The Hydrographic Winch.

This was supplied by Messrs. Elliott & Garrood, of Beccles, and installed when the ship was built. Its engine and general arrangement were perfectly satisfactory, but its main drum, intended to take 3000 fathoms of wire, proved too weak for work at depths greater than 2000 fathoms. Being too deep and too narrow in shape, its flanges were not

* Vol. I, No. 1. 'Introduction and List of Stations', by Lt.-Col. R. B. Seymour Sewell, C.I.E., Sc.D., F.R.S., I.M.S.(ret.).

stiff enough to take the very great lateral pressure when full of wire. One flange cracked at Station 22. This was repaired in Karachi, and lasted fairly well, until it cracked again at Station 127. For many stations we were compelled to use the small drum of the big trawling winch. This worked well, if rather slowly, and had the advantage that the lead for the wire was more convenient.

(*b*) The Wire.

The wire used was 4 mm. in diameter and made of hardened spring steel. In all we had some 8000 fathoms, most of which consisted of six strands, each made up of seven wires. One length was of an extra flexible type consisting of six strands each of nineteen much finer wires, and was much easier to splice and pleasanter to work with, and seemed to wear equally well. The wire was greased both when running out and when being hauled in, on the earlier cruises with a heavy motor-gear oil, and on the latter ones with a castor oil and tallow mixture used for the trawl warp. What rusting occurred was almost entirely superficial, and no trouble was experienced from this cause.

(*c*) Blocks.

We had at first no special blocks for the hydrographic wire and used ordinary small iron blocks. These caused considerable trouble, the wire cutting rapidly into the cast-iron sheaves. Thus it twice happened that a sheave collapsed while in use. Later a special block was made for us by Messrs. R. W. Munro & Co. with a heavy bronze sheave, some 8 inches in diameter, mounted on ball bearings, fitted with " Tecalemit " greasing nipples. This block worked perfectly even at the highest speeds, and we had no further trouble from this cause.

The wire was led straight forward from the winch, through this special block suitably placed, then through a spring-loaded " accumulator " mounted on a davit, and finally out over the measuring sheave. This last was simply a large ball-bearing block containing a sheave 1 metre in circumference coupled to a revolution counter. The accumulator served to smooth out the shocks which would have been caused by the rolling of the ship and any unevenness in the running of the winch. It was especially useful in indicating when bottom had been struck with the heavy Bigelow sounding tube.

(*d*) Water Bottles.

For the shallower depths down to 150, and occasionally 400 metres, the standard Nansen-Pettersson insulating bottle was used, and worked perfectly satisfactorily. It was, however, found to be considerably slower in use than the reversing bottles, and its special valve was much less convenient for emptying than a simple tap would have been.

All the deeper samples were taken with Ekman-pattern reversing bottles of various types. The majority had been used on Sir Douglas Mawson's expeditions in the " Discovery " to the Antarctic, and were made by Messrs. Elliott & Garrood. The end plates, springs, and the insides of the barrels of three of them were plated with chromium before the Expedition in order to obtain samples uncontaminated with copper from the bottle. The chromium proved extremely resistant to sea-water. A further three bottles were specially modified by Messrs. Munro to take three instead of two thermometers, in

order to allow of the addition of one unprotected to the usual two protected ones. All these suffered from the slight defect that the bottle usually failed to lock into position in the frame when closed under water. This made it necessary to haul the bottle slowly through the water when heaving in, to avoid disturbing the thermometers. The defect appeared to be mainly a matter of balance, since bottles obtained later from Messrs. Munro, which were fitted with balance weights, hardly ever failed to work perfectly.

Of " messengers " two types were used. In one the top movable portion was locked in either the open or the closed position by a spring-loaded plunger; in the other it was simply held in the closed position by a spring. The latter type proved the more convenient in use, since it could be operated with one hand.

(*e*) Thermometers.

The number of these used has already been recorded (Vol. I, p. 11). Nansen-Pettersson insulating bottles were used at most stations down to 150 metres, and in a few cases deeper. They were fitted with Schmidt protected non-reversing thermometers, which were found to be extremely satisfactory. Correction for adiabatic cooling has been applied in the few cases where it was significant. Owing to the rapid change of temperature with depth and the small temperature effect of the pressure at 150 metres, this was only rarely the case. Occasionally the temperature as determined in this way was checked by using reversing thermometers at the same depth; the results were in excellent agreement.

At greater depths protected reversing thermometers supplied by Negretti & Zambra were used, usually in pairs, while the lowest bottle of each haul carried in addition an unprotected thermometer for correcting the depth of observation. The readings of the protected thermometers have been corrected according to the formula and tables of Subow (1931), while those of the unprotected thermometers have been treated by the formula of Sverdrup (1933). Paired thermometers gave good agreement, the difference rarely exceeding 0·01° C. All thermometers were standardized by the National Physical Laboratory both before and after the expedition.

METHODS OF ANALYSIS.

(*a*) Salinity.

The halogen content of water samples was determined by the method of Oxner and Knudsen (1920). After a little practice this method can be used with machine-like precision and speed. Frequent titrations of " normal sea-water " supplied by Knudsen were used as a check, and duplicate samples analysed. It was found that a blue " daylight " bulb greatly increased the ease of determining the end-point of the titration. The more mechanical the method became, the more accurate were the results obtained.

The titration values were corrected to halogen, and these to salinity and σ_o by the tables of Knudsen (1901); σ_t was calculated from the tables of Matthews (1932). The calculation of anomalies for dynamical computation of water movement was made from the tables of Sverdrup (1933*b*).

(*b*) Oxygen.

Dissolved oxygen was determined by a modification of Winkler's method described by Jacobsen and Knudsen (1921). Manganous sulphate was used instead of the usual chloride, and was found to be less contaminated with iron. The sodium thiosulphate used for the titration was kept as a normal solution and diluted as required. The diluted solution (N/200) was allowed to stand, and then standardized by the iodate method (Alsterberg, 1926). Standardizations were repeated at frequent intervals, but it was found that the thiosulphate so prepared was remarkably stable, a variation of more than 0·2% in two days being very unusual, while this limit was never reached in a single day. At first some trouble was experienced owing to the " drift " of the end-point, but it was soon found that as the method became thoroughly mechanical this error was entirely eliminated. An ordinary electric bulb and a white porcelain plate under the bottle used for the titration was found better than daylight to work by. For collecting the samples glass bottles were used whose stoppers had been ground off at an angle, and a little experience with these made it quite easy to avoid enclosing bubbles of air. The bottles held approximately 120 ml., but their volumes were not determined ; instead, samples of 50 ml. were withdrawn with a pipette for titration. This method saves a great deal of trouble in the calculations, and enables duplicate titrations to be made if desired.

The rather sudden and sometimes erratic variations in the amount of dissolved oxygen found led us to suspect the accuracy of our results, but it was found that duplicate samples gave results agreeing within the error of the titration. Even samples collected on different hydrographic hauls but from the same depth at the same station showed no significant disagreement. We therefore reached the conclusion that the methods of collection, bottling and determination were not at fault, but that oxygen does indeed vary in a rather erratic manner, probably on account of its biological reactions.

The percentage saturation was calculated from the salinity and temperature data with the aid of the table of Subow (1931).

For the determination of phosphate, nitrite and silicate a long tube-colorimeter of the type described by Sund (1931) was used. This instrument was modified by the substitution for its original 2-volt lamp of a 40-watt " daylight " lamp run from the ship's electrical system, and by the addition of an extra sheet of opal glass to the diffusing system. Of the two pairs of wedges supplied the cemented ones were the more satisfactory, as the built-up ones defied nearly all attempts to prevent their leaking. For nitrate estimations a Duboscq-pattern colorimeter by Leitz was used.

(*c*) Phosphate.

Phosphate was determined by the method described by Sund (1931) and his colorimeter was used for the comparisons. The green colour frequently reported in operating this method did not appear often, but fading gave considerable trouble, probably on account of the high working temperatures. The difficulty of fading can be largely eliminated by standardizing the method both in the mixing and in the time allowed to elapse before comparison.

(*d*) Silicate.

Samples were collected in waxed bottles and analysed, as described by Atkins (1926). The picric acid solution used as a standard had been previously standardized against a solution of known silicate content at the Plymouth Marine Biological Laboratory through the kindness of Dr. L. H. N. Cooper. In water from depths not exceeding a few hundred metres no difficulty was found with the determination. In such samples the colour of the picric acid solution is always slightly greener than that developed by the silicate. In some deep samples, however, usually of the order of 2000 metres, this colour difference was reversed, the picric acid solution being the yellower of the two. It is hoped to investigate this phenomenon later; at present the only suggestion that can be made is that it is due to the presence in the samples of some other substance such as iron.

(*e*) Nitrate, Nitrite and Ammonia.

The methods used were essentially those described by Wattenberg (1931) and Witting (1914), and are considered with the work of Mr. Gilson on the nitrogen cycle (Section 2 of this volume).

(*f*) Hydrogen-ion Concentration.

This was determined by means of indicators and buffers, and the method will be discussed by Mr. Mohamed in his section on the subject. The results given in the tables have been corrected for salinity and temperature as described in that section.

ACKNOWLEDGMENTS.

It cannot be too strongly emphasized that this volume is the work of three persons, and the authors wish to give full credit to their colleague Mr. Mohamed. As well we wish to thank the whole ship's company for unfailing assistance.

The services rendered by the Hydrographic Department of the Admiralty were very great. Through the courtesy of the Hydrographer of the Navy, H.M.S.S. "Endeavour" worked several stations for us in the neighbourhood of Ceylon; Mr. D. J. Matthews also assisted wherever possible, both with advice and practical collaboration; lastly we have to acknowledge the loan to us of Lt.-Cmdr. W. I. Farquharson, R.N., to whose care and precision we owe the accuracy of our station positions and depth records, and to whom we are indebted for advice on innumerable points.

The Meteorological Office very kindly arranged for the collection of many surface samples and temperatures by ships of the Merchant Service, and we wish to record our gratitude to the officers who performed these duties so ably.

Through the courtesy of the "Discovery" Committee R.R.S. "Discovery II" carried out observations at a series of stations off the African coast, and for these we wish to thank both the committee and the officers who performed the work.

The kindnesses of the staff of the Plymouth Marine Biological Laboratory have been too numerous for detailed enumeration, but in particular we must thank Dr. E. J. Allen, C.B.E., F.R.S., Dr. W. R. G. Atkins, O.B.E., F.R.S., Dr. L. H. N. Cooper, Mr. H. W. Harvey and Mr. G. A. Steven for help both before and after the Expedition.

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For innumerable courtesies in Egypt we wish to acknowledge our gratitude to the Egyptian Coastguard Service and the Faculty of Science in the Egyptian University. Particularly helpful were Professor D. H. Bangham and Dr. H. Faouzi.

Finally and above all we wish to thank the John Murray Committee, and especially Professor J. Stanley Gardiner, without whose untiring efforts the Expedition would have been impossible.

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THE NITROGEN CYCLE

BY

H. CARY GILSON, M.A.

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I. INTRODUCTION.

One of the greatest difficulties in the study of the relation between marine organisms and their environment is the extremely close interrelation between biological factors. We cannot fully understand the plants without knowing all about the animals which feed on them, and the bacteria which dispose of their dead remains, any more than we can understand the animals apart from the plants and bacteria which form their food, or

the bacteria apart from the plant and animal residues which provide their energy. It was thus found impossible to study the nitrogen cycle without preliminary consideration of the factors which govern the productivity of the sea as a whole, and this forms the subject of the next section.

Sections III and IV represent attempts to approach the nitrogen cycle itself from two distinct points of view, the one by the method of laboratory experiment, and the other by the investigation of the conditions actually obtaining in the sea. For the latter, which came first in time, I was fortunate to have the opportunity of the cruise of the John Murray Expedition (in H.E.M.S. " Mabahiss ") in 1933–34, and I am much indebted to Professor Stanley Gardiner for suggesting that I should undertake the study of the distribution of nitrogen compounds in the area investigated. After the return of the expedition, examination of the data obtained suggested certain problems, especially regarding the distribution of nitrite, which seemed as though they would repay investigation by laboratory experiments. With the kind permission of the Murray Trustees and of the authorities of Trinity College, Cambridge, these experiments were undertaken at the laboratory of the Marine Biological Association at Plymouth while holding the Cambridge University table there during the first four months of 1935. They were in some degree successful, and the results are reported in Section III. They have been put before the account of the work carried out during the John Murray Expedition because they help to some extent in elucidating it. As was bound to be the case in the short time available, they are only of a preliminary nature, and were intended partly to determine whether the method was in fact likely to yield results of value. They have shown the necessity for modifications, especially in regard to the part played by bacteria, but they have also shown that it is possible to obtain much information about the metabolism of phytoplankton organisms by the chemical analysis of cultures.

It will be observed in Section IV that the aspect most stressed is the phytoplankton. As the primary producers of organized living matter from inorganic materials, the photosynthetic forms of life are the logical starting-point for the investigation of the cycle. It is therefore from this point of view, rather than that of the oceanic circulation of water, that the John Murray Expedition results have been treated here. Similarly in all questions of productivity, it is the production of phytoplankton which has been primarily considered.

Finally in Section V an attempt has been made to survey present-day knowledge of the nitrogen cycle, especially the bacterial side of it, on the basis of recorded work both in the laboratory and at sea, and to draw such conclusions as are possible from the available data.

Quantitative investigations of the plankton were started by Hensen (1887), who was the first to show the seasonal variations which are now a commonplace of all investigations in temperate regions. Then the plankton expeditions (*vide* Hensen, 1887 ; Gran, 1912 ; Lohmann, 1920) demonstrated the far denser plankton population of coastal waters as compared with open sea. Two great problems had now presented themselves —the reasons for the greater productivity of coastal waters, and the factors controlling the spring plankton outburst, and almost all subsequent research on the subject has been directed towards the solution of them.

The earlier work was all done with nets of various types, and it was not until Lohmann introduced the use of the centrifuge that the importance of the very small organisms,

which escape even the finest nets, was realized. The method has been used since, especially by Gran and his collaborators, and has produced excellent results. It has the great advantage of giving an accurate estimation of the population density in the water at a known depth, but owing to the small volume of samples which can be examined, it does not always give a representative picture, if the plankton is sparse. The net has the advantage of filtering a larger volume of water, but there is always the difficulty of determining either the volume filtered, or the depth from which the contents came. It is the only method available for the larger zooplankton, and must in many cases be used in conjunction with the centrifuge. An improvement recently devised by Harvey (1934*a*) consists of an instrument mounted in the mouth of a fine silk net, which measures the volume of water which passes through the net. This, combined with the method used by Kreps and Verjbinskaya (1930) (see also Harvey, 1934*a*) of estimating the phytoplankton by its pigment content, provides a very useful rapid method of determining approximately the total phytoplankton content of the water. Another improved form of the larger net is the serial closing net of which a set is used on the American research vessel " Atlantis " (Clarke, 1933).

An important contribution to the problem was made by Brandt (1899, 1902, 1920), who showed that the nitrogen, and especially the phosphorus compounds, which are essential for the growth of the phytoplankton, are often present in sea-water in extremely minute amounts. He put forward the theory that the reason for the richness of certain areas and certain times of the year might be the presence of an abundant supply of these nutrient salts, which in other places and at other seasons might be available only in very small quantities, insufficient to support a dense plankton population. This theory, as we shall see, has been amply confirmed by subsequent work.

Brandt and Raben's work showed the value of chemical investigations to elucidate planktological work. It was, however, soon realized that the methods they used were not sufficiently sensitive for the purpose, and were far too laborious to allow of the accumulation of any great mass of data. Thus no great progress was made until a new phase was begun by the introduction by Atkins and Harvey of improved methods for the estimation of the very small quantities of phosphates and nitrates often present in sea-water. The results obtained at Plymouth from 1922 onwards (Atkins, 1923, etc. ; Harvey, 1926, 1928*a* ; Cooper, 1933) showed for the first time in a really satisfactory manner that there is a regular cycle in the amounts of nutrient salts present in the sea, which corresponds with the annual cycle of the phytoplankton. The great importance of combining chemical analysis of the water with counts of the plankton population is well shown by Braarud and Klem (1931), who reached considerably different conclusions from those previously drawn by Gran (1923, 1927, 1929*b*, etc.) and his colleagues from data obtained in the same area on the phytoplankton distribution alone.

The emphasis in recent years has thus been rather on the chemical aspect of the problem. This is well brought out by a comparison of the work done on the " Michael Sars " expedition in 1910 with that done on the " Meteor " expedition of 1925–28.* Since the chemical method of approach still seemed the most likely to yield valuable results, the same is true of my own work on the John Murray Expedition. There I was allotted the task of carrying out all the analyses for nitrate and nitrite on the samples

* See Murray and Hjort's book, ' Depths of the Ocean ', and the " Meteor " reports still in process of publication.

collected. The expedition also had constructed a quantitative plankton net of the type designed by Harvey (1934*a*), of which I was put in charge.

I am much indebted to Dr. L. H. N. Cooper for help with the analytical methods and preparation of reagents before the expedition set out; to Dr. E. J. Allen, C.B.E., F.R.S., for help and instruction in the methods of culturing diatoms; and to Mr. H. W. Harvey for advice with regard to the design and operation of the quantitative plankton net.

II. THE CONDITIONS FOR THE PRODUCTION OF PLANKTON.

This whole question has been so frequently reviewed that there is no need to go into the data in any great detail. It is, however, necessary for the understanding of what follows to sum up briefly our present knowledge on the subject.

Since Brandt's (1905) first review of the problem the outlook has changed much. At that time the only knowledge available was of the actual distribution of plankton, and it is only in more recent years that improved methods of analysis have given the mass of chemical data now available. The theory he developed in his series of papers (1899, 1902, 1920), " Über den Stoffwechsel im Meere ", that production was closely dependent on supplies of nutrient salts has been abundantly verified, and was again reviewed by him in 1929. This theory forms the basis of all modern work, and has been brought more up to date by Gran (1931).

(*a*) Physical Factors.

The most important of these for the phytoplankton is light. Owing to absorption by the water and scattering by the particles suspended in it, the illumination falls off rapidly below the surface of the sea, so that there is only a comparatively narrow zone in which light is sufficient for photosynthesis. The thickness of this layer varies greatly with weather, season, and latitude, from *nil* in the polar winter to 100 metres or more in clear water and low latitudes.

Gaarder and Gran (1927) observed the changes in oxygen concentration in flasks of sea-water rich in phytoplankton suspended at various depths. This enabled them to determine the depth at which the oxygen produced by photosynthesis just balanced that consumed by respiration. This level, called the " compensation point ", marks the lower limit of the zone in which effective phytoplankton growth is possible; in Oslo Fjord at the time of the spring diatom outburst it lay about 10 metres below the surface. Using diatom cultures instead of raw sea-water Marshall and Orr (1928) carried out similar experiments in Loch Striven, where they found the compensation depth to vary from 2 metres on a dark day in mid-winter to 30 metres on a bright day in summer.

The relation to light is complicated by the fact, first observed by Schimper (Karsten, 1905), that too much light is injurious to diatoms. This was verified by Marshall and Orr, who found that even in winter illumination is supra-optimal at the surface on a sunny day, while in summer the deleterious effect may at times extend 2 or 3 metres below the surface. Flagellates and Dinoflagellates are apparently not injured in this way by excessive light (Gaarder and Gran, 1927). Jenkin's* similar experiments in conjunction with measurements of the illumination show that below the limit of this injury effect, photosynthesis bears a direct relation to the amount of light reaching any given level.

* Personal communication from Miss P. M. Jenkin, whose paper is in preparation.

Atkins (1928) (*cf.* also Herdman, Scott and Dakin, 1910) suggested that in the winter in temperate regions lack of light limits the growth of phytoplankton, and that the spring outburst is conditioned by the increased amount of sunshine in March. Marshall and Orr (1928), on the basis of their experiments, consider this unlikely. They stress the great importance of length of day, and while admitting that light may be limiting in mid-winter, they remark (p. 340), ". . . it is difficult to believe that in the lengthening days of February and March light can act as a limiting factor for growth in the sea". The same view is expressed by Føyn (1929), on the ground that Atkins's theory does not explain the fact that the outburst on the Norwegian Coast begins exclusively near land. On the other hand Braarud and Klem (1931) have shown that there is no foundation in fact for the view put forward by Føyn and by Gran that the outburst in Norwegian coastal waters is conditioned by an increase in the supply of nutrient salts from land. They therefore return to the theory that the increasing amount of light in spring is one of the determining factors.

At the present time it is impossible to say whether this view is correct or not. The influence of illumination is much complicated by the fact that both sunshine and length of day have to be taken into account, and by the effect of stormy weather, which greatly increases the loss of light at the water surface. That light is not always the key factor is shown by the fact that the spring maximum occurs about the same time in the Gulf of Maine (Bigelow, 1926) as at Lofoten (B. Ruud, 1926) which is 18° farther north. Also W. E. Allen (1927) found no correlation between light and the phytoplankton crop in California.

Whatever the importance of light in relation to the spring outburst, it certainly does limit the thickness of the productive layer in all but very shallow water; its lack is also the reason for the sparse plankton found in winter in high latitudes.

As far as concerns the diatoms, which form the most important element of the temperate phytoplankton, temperature appears to have little influence, abundant growth being possible over a range from $-1{\cdot}5°$ to 30° C. Low temperature never seems to limit production, and indeed the Norwegian workers, Gran, Braarud, Føyn and others, have shown that the spring outburst off their coasts commonly begins when the sea-temperature is at its minimum for the year ($0 - 5°$ C.).

Ostwald (1902) has pointed out that a low temperature may favour the plankton owing to the higher viscosity of the water, which enables the organisms to remain for a longer period in the photosynthetic zone. The importance of viscosity is very hard to estimate. That diatoms do sink after a period of rapid growth has been observed by Marshall and Orr (1927), by Lohmann (1908), and by Gran (1915), who suggested that the increased rate of multiplication at a higher temperature might balance the increased rate of sinking. In the absence of any culture experiments in which illumination and temperature have been controlled, it is impossible to judge of the validity of this view. That sinking below the limit of adequate illumination is not the only, or indeed the main cause of the end of an outburst, is shown by the fact that similar sinking coincides with a fall in the growth-rate of a culture, where the illumination is unaffected (see below, p. 38).

Although temperature does not appear ever to limit the production of plankton, it undoubtedly does affect its composition. Some species of diatom are judged from their occurrence to be thermophile; the Dinoflagellates in temperate waters always have their

maximum in the warmer conditions of July and August.* Conversely, the Coccolithophoridæ, so characteristic of tropical oceans, are never found where the temperature is low; thus in the Gulf of Maine Braarud (1934) records that *Pontosphæra huxleyi* was confined to water with a temperature above 10° C.

By far the most important effect of temperature is its indirect one on the density of the water. The difference in density between the surface layer warmed by the sun and the cooler water below prevents mixing across the boundary, so that the surface layer is cut off from all possibility of renewal of its nutrient salts from the rich stores in the deeper water. As may be clearly seen in the stations worked in the Gulf of Aden by the John Murray Expedition, and described below, such a boundary may also prevent the utilization of the nutrient salts in the water immediately below it, even though the illumination may be adequate. This is due to the growing cells being held up at the boundary, unable to sink below it until after their active life is over.

On the other hand, the development of stably stratified conditions may have an important effect in promoting the growth of the plankton. As we shall see in discussing the chemical factors, vertical mixing at some time of the year is often the most important factor in determining the productivity of an area. While active mixing is in progress, no great plankton development can occur, because the growing diatoms are so rapidly carried down below the limit of adequate illumination; thus a sufficient stock can never be accumulated near the surface to counteract wastage, and start a vigorous production. This effect appears both off the Norwegian coast (Braarud and Klem, 1931), and in the Antarctic Ocean (Gran, 1931, and J. T. Ruud, 1930). In both cases the stratification is not due to warming of the surface water, but to the advent of a surface layer of lower salinity, in the first case from the rivers, and in the second from melting ice.

(*b*) Chemical Factors.

Of the many constituents of sea-water the most important for the plankton are the nitrates and phosphates, and it is on the supply of these that the productivity of an area principally depends. Such a supply may be brought to the surface layers in several ways, which produce corresponding variations in the resulting type of plankton production.

During a period of production a continuous hail of dead plankton, both plant and animal, carries both substances down out of the illuminated zone to the bottom, where bacteria return the phosphorus and nitrogen to solution as phosphate and nitrate. In stable water conditions, such as are found nearly everywhere in summer, they remain there as a rich bottom layer. But with the approach of winter in temperate and polar regions, the surface layers of the sea cool, and consequently rise in density. Unstable conditions are thus set up, and helped by the wind, the whole body of water turns over, becoming homogeneous from top to bottom. Owing to insufficient light at this season, the supplies of nutrient salts thus brought up remain unused till the spring. With the

* As pointed out by Gran (1929*b*), their presence at this time of year in conditions which will not support any considerable diatom population is connected with the fact that owing to their mobility and their lower growth-rate (Apstein, 1910), they can make use of far more scanty supplies of nutrient salts. It has been supposed that diatoms and dinoflagellates are mutually antagonistic, but this is not supported by Sleggs (1927). The scarcity of dinoflagellates in spring in European waters is almost certainly due to the low temperature, and not to the presence of abundant diatoms.

increasing daylight of March production begins, and rapidly develops into an enormous outburst, mainly of diatoms, which continues until the end of April. In shallow seas such as the English Channel, where there is considerable tendency for mixing by wind and tide, almost the entire nutrient salt content of the water column can be used up by the plankton. Much of it is consumed in the spring outburst, but considerable production occurs during the summer, until in August the development of a marked thermocline cuts off the supply from below. The rapid fall in the phytoplankton population after the spring outburst is attributed by Harvey (1934*b*) to an increase in the herbivorous zooplankton (see also Harvey, Cooper, Lebour and Russell, 1935).

In very shallow water, such as the Limfjord in Denmark (Ostenfeld, 1913), mixing by convection can occur all the year round owing to heating of the bottom by the sun. Here there is also the possibility of supply of nutrients from land, and a rich plankton therefore persists throughout the summer.

In the deeper water of the Scottish lochs and the Norwegian fjords vertical circulation is only possible for a short time in winter, since the influx of fresh water with the spring rains and the melting of the snows leads to stable conditions early in the year. The supplies brought to the surface by the winter mixing are rapidly used up by the diatom outburst in March and April, and plankton is sparse during the summer and autumn. In some places an irregular supply from land produces a series of minor plankton maxima during the summer months* (Marshall and Orr, 1927 ; Gran, 1929*b*).

In the warmer parts of the world mixing by convection never takes place. In certain areas, however, a continuous supply of nutrient salts is made available by the upward movement of water from the depths. This may occur where a deep oceanic current is deflected by a submarine bank, or where an uneven bottom tends to cause turbulence. The rich plankton produced over the Faeroe Bank from this cause was observed by Paulsen (1909) and Gran (1929*a*), and persists throughout the summer. Similar conditions occur in Puget Sound, where the strong tidal streams running in narrow straits of varying depth result in abundant phosphate and diatoms all through the summer. Alternatively supplies may be brought up by the " upwelling " which occurs along a coast to replace surface water driven out to sea by an off-shore wind. This occurs particularly in the Trade Wind belt on the west coasts of both Africa and America, where it results in continuous rich production. According to Moberg (1928) the plankton off the Californian coast is mostly between 20 and 40 metres down, and he suggests that it is limited above by lack of nitrate and phosphate, and below by lack of light. Hentschel and Wattenberg (1930), in a study of the whole south Atlantic, show how upwelling near the African coast forms small areas which are rich in both phosphate and plankton ; round these are zones still rich in plankton, but in which the phosphate has already been consumed ; west of these again is a large area which is poor in both phosphate and plankton and extends south to about 30° S. Beyond this is another rich area where there is mixing by convection in the winter.

Over most of the tropical oceans, where there is no such source of supply, stable conditions arise, in which production is brought almost to a standstill by the lack of nutrient salts. In the equatorial region the removal of surface water in the currents set up by the trade winds causes a slow upward movement of deep water. Thus the regions

* Harvey (1934*b*) is inclined to attribute the summer fluctuations of the phytoplankton to variations in the proportions of carnivores and herbivores in the zooplankton.

which are poorest in plankton lie from about 10° to about 20° on either side of the equator.

For plant growth another essential, which is present in very minute amounts in sea-water, is iron. Since it is only recently that adequate methods of analysis have been introduced, little is known as yet about its distribution in the sea. Braarud and Klem (1931) found values ranging from 4 to 23 mg. Fe/m^3 off Møre. There the surface water contained more iron than deep water, and the fjord more than the sea outside, owing to the influx from a river. Some of the iron is retained by a Gooch crucible, and evidence is put forward that some of it is in the form of colloidal ferric hydroxide.

Thompson, Bremner and Jamieson (1932) found on the other hand that the iron content rose with depth. The amount present in the San Juan Archipelago region ranges from 60 mg. Fe/m^3 in March and April to a minimum of 32 mg. Fe/m^3 in July. Lower concentrations were found in the photosynthetic zone, indicating its consumption by the plankton. In a later paper (Thompson and Bremner, 1935) the iron content of the open water beyond the continental shelf is reported to range from 11 to 45 mg. Fe/m^3. Of this about half is in an " insoluble " form retained by fine grain filter-paper. According to Cooper (1935*b*) an even smaller proportion (probably not more than 2 mg. Fe/m^3) is in true solution and capable of passing a membrane filter. He also reports that the iron content of phytoplankton is approximately four times the phosphorus content, so that the demand for iron during an outburst must be very great. Thus on one occasion as much as 16% of the total iron present was in the plankton. Since according to his calculations the solubilities of ferrous and ferric ions in sea-water are both exceedingly small (of the order of $3 \cdot 5$ and 10^{-12} mg. Fe/m^3 respectively), it is difficult to see how the plankton organisms obtain their supplies. Evidently much of the iron present must be in the form of stable complexes, such as ferri-fluoride, and a further large fraction is apparently in a colloid-disperse state.

Of the source of supply very little is known. Considerable quantities, mostly in an insoluble form, may be brought down by rivers ; 67 mg. Fe/m^3 were found in the waters of the Rauma by Braarud and Klem, and 1250 mg. Fe/m^3 in surface water near the mouth of the Fraser River by Thompson and Bremner. Most of this is probably in the form of the basic carbonate. Some may go into solution and become available for the phyto-plankton, but much of it probably sinks unchanged to the bottom. In any case most of it is probably hydrolysed sooner or later to the insoluble hydroxide, and is thus permanently removed from the circulation. Whether any iron is brought up from the depths with the nitrate and phosphate is unknown, but the high iron content of many deep-sea deposits makes this seem unlikely. It is possible that we have a clue to the source of supply in Cooper's (1935*b*) observation that the iron content of zooplankton is very much less than that of phytoplankton. This corresponds with his further observation that animal fæcal pellets are especially rich in iron, containing about five times as much iron as phosphorus. It is therefore probable that the iron is returned to circulation more rapidly than is the phosphorus, and so may complete the cycle more than once in the year, as is apparently the case with silica (Cooper, 1933). It is thus possible that the apparently inadequate supplies may yet suffice for the requirements of the plankton.

The importance of iron to the plant is connected with the formation of chlorophyll. Thus Emerson (1929) and Fleischer (1935) varied the chlorophyll content of *Chlorella vulgaris* by varying the iron content of the culture medium. In culture experiments with

raw plankton (Gran, 1931, 1933), the addition of iron in various forms usually gave better growth than controls enriched with nitrate and phosphate only, especially in the case of neritic species of diatom grown in oceanic water. Similarly Harvey (1933), working with cultures of *Nitzschia closterium*, found that the addition of 1 mg. Fe/m^3, as ammonium ferri-citrate, approximately doubled the growth-rate. The ferri-citrate complex is not apparently immediately available to the diatoms, and it is suggested that they depend on ions given off " which escape hydrolysis to the unavailable hydroxide ". Analysis failed to detect any ferrous or ferric ions until the sea-water had been acidified and vigorously oxidized, and Harvey concludes that there is probably less than 1 mg. Fe/m^3 present in a form immediately available to the plankton, though more apparently becomes available as this is consumed.

The question of the effect of iron is bound up with the fact, long known, that the growth of various plants and algæ in culture is accelerated by the addition of an extract of soil or vegetable matter (E. J. Allen, Miquel, Schreiber and others). This has been supposed to be due to an addition to the available iron supply. However, Harvey (1933) showed that even if soil extract does add to the available iron, which is not always certainly the case, it also has some other independent stimulant effect. This is supported by the observation that an alkaline extract of soil is more effective than an acid one, Evaporation and ashing of the extract destroyed its growth-promoting properties, and Harvey suggests that these depend on some organic compound.

The isolation and synthesis of a plant growth substance or " auxine " (β-indolyl-acetic acid) by Kogl, Haagen-Smit and Erxleben (1934) suggests that this may be the compound concerned. This substance is known (Kogl and Haagen-Smit, 1931 ; Kogl, 1933) to be present in urine, and accordingly, at Mr. Harvey's suggestion, an experiment was tried to test this. A series of cultures of *Nitzschia closterium* abundantly supplied with nitrate and phosphate gave considerably more rapid growth in those flasks to which 0·1% of filtered and sterilized urine had been added, but the experiment was only of a preliminary nature and cannot be regarded as conclusive.

There remains one other chemical property of the water, which may influence the growth of phytoplankton. Barnes (1932) found that *Spirogyra* grew more rapidly in water which had been recently frozen than in water which had just been distilled, or had been long in the liquid state. He suggested that the trihydrone molecules present in the ice-water stimulated growth. This was tested on diatoms by Harvey (1933) with positive results in most, though not in all, experiments.

It is possible that any or all of these last three factors—iron, auxine, and trihydrone—may be the explanation of the apparently greater productivity of coastal waters as compared with the open ocean. This may, no doubt, be partly explained by the more effective winter mixing of the shallower water, but the nutrient salt supply is not a complete explanation of the phenomena of the Norwegian coast. It has been repeatedly observed (B. Ruud, 1926 ; Føyn, 1929 ; Gran, 1929*b*) that production starts close to the shore, and spreads out over the banks. This was attributed to the influx of water from land, which occurs with the melting of the snow. Braarud and Klem (1931) showed that the addition of nutrient salts from land by this means is insignificant, but their criticism that the big floods due to melting snow do not occur till May, when the plankton outburst is over, is not really to the point. There remains the possibility that the outburst is started off by the snow-water from low-lying land, as suggested by Gran (1923), not in

virtue of its content of nutrient salts, but because it brings trihydrone, iron, or auxine, or all three. There is also the probability already mentioned (p. 26) that the fresh water helps by promoting stability. Any one of these factors might be sufficient to explain the observed facts, but it is not unlikely that all three play a part in giving the fillip needed to start production. Once started, the outburst develops extremely rapidly in the favourable conditions, since growth is approximately logarithmic at first.

Owing to the abundant plankton found there in spring and summer, when most of the observations have been made, it has been generally supposed that the total annual production is much greater in high latitudes than in the tropics. On the basis of calculations concerning the western part of the tropical Atlantic, Seiwell (1935*a*) has thrown doubt on this view.

In the English Channel Atkins (1923) estimated from the phosphate consumption that the minimum total annual production was 1·4 kg. (wet weight) of phytoplankton per square metre of sea surface. Cooper (1933*a*) reached closely similar figures from the changes in CO_2, oxygen, phosphate and nitrate. Kreps and Verjbinskaya (1932), again on a phosphate basis, calculated the minimal production in the Barents Sea to be between 2·9 and 5·6 kg. per square metre for the 100-day season. Seiwell's figure, recalculated on the same basis, is 4·64 kg. per. sq. m. for the year, and he concludes that the area is more productive than anything found in higher latitudes, except perhaps the Arctic regions. This is attributed to the fact that production continues at an approximately uniform rate throughout the year. Comparison of this rate with those reported by various observers at the time of the northern spring outburst is interesting (Table I) :

TABLE I.

Author.	Region.	Method of estimation.	Production rate. (grm./m²/day [wet wt.]).
Atkins (1923)	English Channel	CO_2 consumed	8·3
Cooper (1933*b*)	,, ,,	Various	8·3
Marshall and Orr(1927)	Clyde	O_2 produced	26·6
Gran (1927)	Bergen	,,	6·7
,, ,,	(Maximum figure in cultures)		25
Gran (1929)	Romsdalsfjord	O_2 produced	2·14–9·94 (5 stations).
Føyn (1929)	Lofoten	,,	18·6
Kreps and Verjbinskaya (1932)	Barents Sea	P consumed	29–56
Seiwell (1935)	Tropical Atlantic	O_2 consumed	12·7

It will be seen that although his area is one which was found by Hentschel and Wattenberg (1930) to be distinctly poor in plankton, Seiwell's figure is higher than most of the estimates for the vigorous spring production in the English Channel and off the Norwegian coast.

There are several reasons for the discrepancy. Seiwell's estimate is based on a calculation of the annual oxygen consumption in the whole water column down to 2000 metres, especially the "oxygen-poor layer" between about 200 and 1200 metres (Seiwell, 1934). It is assumed that the oxygen so consumed represents the oxidation of the annual

crop of organic matter produced in the region in question. Now the water of the oxygen-poor layer is water which left the surface in high latitudes beyond 40° S. or 50° N., and is moving towards latitude 15° N. at about 200 miles per year. The assumption, therefore, that the oxidizable organic matter present has originated in the region under discussion seems a little doubtful. We have no data on the time taken for the complete breakdown of organic matter in the sea. The English Channel data, showing apparently complete regeneration of phosphate and nitrate each autumn, are inconclusive, since regeneration is continuous at the bottom, and we have no means of knowing that the phosphorus and nitrogen liberated each autumn is the same as that which was consumed in the surface layers during the preceding summer ; there may be several years' stores awaiting bacterial action on the bottom. However, even if, as is probable,* regeneration is completed within the year under those conditions, it does not follow that it is equally rapid in the water under consideration here. The regeneration process is known to be most rapid near the bottom, and as the water on which Seiwell's calculation is based has never been in contact with the bottom, decomposition may be very much slower. It is thus possible that a proportion at least of the organic matter oxidized has come with the water from elsewhere.

Another assumption involved in the calculation is that all the oxygen consumed has been used in the oxidation of carbon. This is certainly not the case, and in calculating the organic matter represented, allowance should be made for the oxygen consumed in oxidizing ammonia to nitrate. Since—

$$NH_3 + 2O_2 = HNO_3 + H_2O,$$

each atom of nitrogen requires 2 molecules of oxygen, whereas each atom of carbon only requires 1 molecule. Now plankton contains approximately 1 atom of nitrogen for every 7 of carbon, so that approximately 20% of the oxygen consumed is required for the oxidation of nitrogen compounds. The relation is only an approximation, since we cannot be certain that all the combined nitrogen is converted to nitrate at the same time as the carbon is oxidized to CO_2. If we apply this correction to Seiwell's figures we obtain 3·7 kg. (wet weight) per square metre as the total annual crop, and 10·2 gm./sq. m. for the daily production.

This is still unexpectedly high. The reason is probably that the figures quoted for more northern latitudes are all too low. Seiwell's method of calculation from the oxygen consumption gives a maximal value for the organic production. All the other methods—oxygen production, CO_2 consumption, and phosphate consumption—give minimal estimates, since no allowance is made for simultaneous oxygen consumption (by zooplankton, bacteria, and by the phytoplankton itself) or regeneration.† It has been suggested (Føyn, 1929) that the resulting estimate of the total crop may be as much as 100% too low, and Gran's (1927) figures for the production in cultures tend to support this view.

Since the data for the two regions is by no means comparable, the comparison between the two is of doubtful value. We are, however, probably justified in concluding that, while the area investigated by Seiwell, which is fairly typical of tropical oceans, is rather

* Cooper (1935*a*) finds that phosphate is rapidly and completely liberated from zooplankton material *in vitro*, while the process is very much slower and incomplete in the case of phytoplankton.

† A tentative correction for regeneration has been applied by Kreps and Verjbinskaya to their figure quoted above.

more productive than has been commonly supposed,* it is considerably less so than the rich areas off the Norwegian coast and in the polar oceans, and not very different from the English Channel. Probably the most productive regions of all are the upwelling areas in the tropics.

III. CULTURE EXPERIMENTS.

An attempt was made to gain some insight into the changes to be expected in the sea by studying the metabolism of phytoplankton organisms, using " persistent cultures "† of plankton diatoms (Allen and Nelson, 1910 ; E. J. Allen, 1914 ; Schreiber, 1928).

Some cultures were obtained by picking out small numbers of cells or chains from " very fine " tow-nettings with fine sterile pipettes under a binocular dissecting microscope. These were washed in one or more watch-glasses of sterile sea-water, and then transferred to a flask of culture medium. Another method used is to " plate " 0·5 to 1·0 ml. of tow-netting in about 20 ml. of culture medium in a Petri dish, which is then placed in a good diffuse light in a place free from vibration. Examination under the binocular microscope after 3 or 4 days will usually show a number of small colonies of diatoms, which can be picked out with pipettes, and transferred to culture flasks. (For further remarks on this method see Allen and Nelson, 1910).

By these methods the following species were obtained in culture between January and April, 1935 :

Biddulphia regia M. Schultze.
Asterionella japonica Cleve & Möller.
Coscinodiscus subbulliens Jörgensen.
Ditylium Brightwelli (West).
Lauderia borealis Gran.
Chætoceros curvisetus Cleve.
C. affinis Lauder.
C. teres Cleve.
C. decipiens Cleve.

Of these, *Biddulphia regia*, *Lauderia borealis*, *Chætoceros curvisetus* and *C. decipiens* were found the most suitable for the purpose of experiment, being vigorous growers and fairly easily counted. *Asterionella japonica* also grew well, but proved impossible to count satisfactorily. *Ditylium Brightwelli* would have been an admirable subject, but the culture was obtained too late for any use to be made of it.

The cultures were grown in a north window, the row of flasks resting on a strip of mirror to give better utilization of the rather scanty winter light. Under these conditions, even in the short days of January, growth rates of the order of one division in 48 hours were observed at a temperature of 10–12° C. In the better light of March and April, when the temperature had risen somewhat (12–15° C.), growth was very much more rapid, and subculturing every three or four days was required to prevent the cultures becoming too dense and unhealthy.

* Seiwell's (1935*b*) recent calculations of the eddy transfer of phosphate through the thermocline, which have just come to my notice, help to explain this, and show that the discontinuity layer is not so complete a barrier as it has been thought to be.

† " Persistent culture " is the term used by E. J. Allen to describe a culture which will give healthy subcultures and contains one species of diatom and no other organisms except bacteria, which are always present.

The culture medium found most satisfactory was a modified "Miquel sea-water" (Allen and Nelson, 1910), in which nitrate was reduced to give the ratio $N : P_2O_5 =$ approximately 2·5 : 1. Schreiber (1928) remarks that excess nitrate tends to favour the bacteria, and there is no obvious advantage to the diatoms in the enormous excess of nitrate in the original medium. Clean sea-water passed through a Berkfeld filter, enriched with suitable amounts of nitrate, phosphate and iron and sterilized, was tried, but did not give such satisfactory cultures. This is in agreement with E. J. Allen's (1914) findings, where he suggests that the precipitate formed by the Miquel solution has some purifying effect on the water, which makes it a more favourable medium for the growth of diatoms.

The small stock cultures (50–100 ml.) were far too small for experimental purposes, and the Miquel culture solution is too rich in nitrate and phosphate to permit of accurate determination of the small changes produced by the growth of the diatoms. Also conditions in these cultures were quite unlike anything ever found in the sea.

For experimental purposes, therefore, 3-litre flasks were filled with clean sea-water, which had been passed through the Berkfeld filter. By blowing alveolar air through the water the pH was brought down to about 7·3, and the water was then sterilized, either by bringing it just to the boil, or by keeping it at 70°–80° C. for at least an hour. The CO_2 lost in this process brought the pH back to the neighbourhood of 7·8. If the preliminary adjustment was omitted, it was found that the pH after sterilizing was usually 8·4–8·5, which is much higher than is ever found in the open sea. After cooling, the flasks were inoculated with from 20 to 50 ml. from a stock culture, the whole well shaken, and a sample immediately withdrawn for counting and analysis. The enrichment added with the inoculation usually brought the nitrate and phosphate of the experimental culture to values just above the winter maxima for the English Channel. Subsequently at varying intervals over periods up to 39 days the cultures were vigorously shaken, and samples of 100 to 150 ml. were poured off. In a portion of this the diatoms were counted; other portions were used for analysis for nitrate, nitrite and phosphate. (For the analytical methods used, see Appendix.)

The results, calculated to show the changes in successive periods, are given in Table II, and the actual changes in the composition of the medium in two typical experiments are shown graphically in Text-fig. 1.

Quantitatively the results are a little disappointing in that the rate of consumption of nutrient salts proved so very variable. This seems to indicate that not too much reliance must be placed on previous calculations based on a single set of analyses (*cf.* Atkins, 1923). The variability of the N : P ratio is probably due partly to the bacteria in the culture, and partly to the fact that nitrate is not the only source of nitrogen available. The fact that there were such large variations in the ratio of nitrogen and phosphorus consumed, as well as in the actual rates of consumption, indicates that the bacteria were probably the primary cause, rather than variations in the rates of consumption by the diatoms.

There remains, however, one observation concerning the nitrogen metabolism of the cultures, which is of great importance, especially in its bearing on the distribution of nitrogen compounds found in the sea. In the earlier stages of the cultures the concentration of nitrite was rather variable, and presumably depends mainly on the balance between the various kinds of bacteria present. But in all cases, when the culture began to die off, large quantities of nitrite appeared. The question of the origin of this nitrite will be

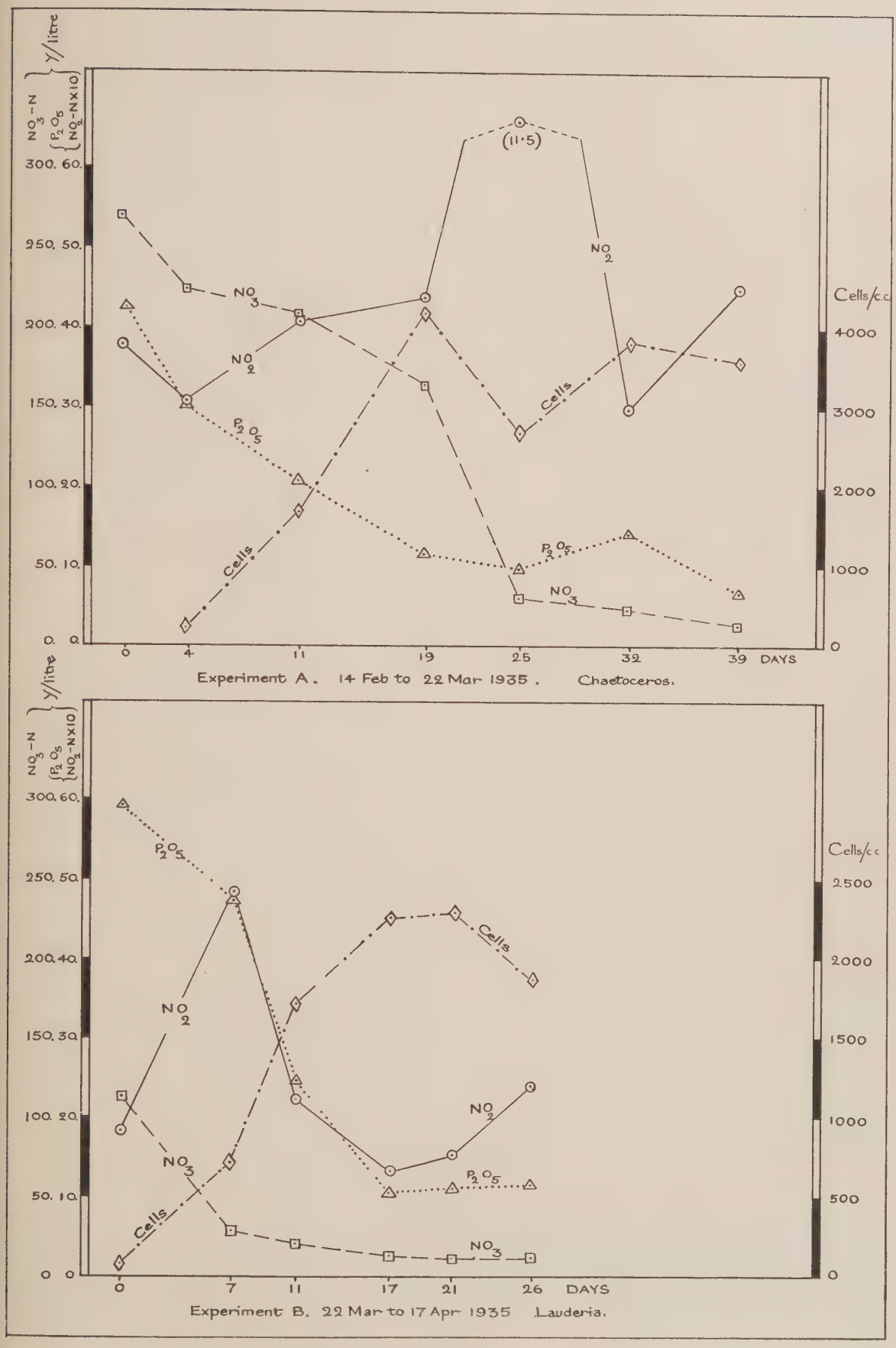
TABLE II.

Period. (Days of experiment.)	Production. (Millions of cells per litre.)	Nitrite. Change in concentration. (γN/litre.*)	Nitrate-N.		Phosphorus.		N:P ratio. (Atoms N per atom P.)
			Fall in concentration (γN/litre.)	Consumption. (γ-atoms per million cells.)	Fall in concentration. (γP_2O_5/litre.)	Consumption. (γ-atoms per million cells.)	
Experiment A. 14.ii to 22.iii.35. *Chætoceros curvisetus* Cleve.							
0–4	..	−0·2	45	..	12·2	..	18·7
4–11	1·45	+1·0	15	0·74	9·8	0·095	7·78
11–19	2·49	+0·3	44	1·26	9·0	0·051	24·7
19–25	(decrease)	+10·1	133·5	..	2·0	..	(338)
25–36	..	−7·0	18·5	..	3·0	..	(31·3)
4–19	3·94	..	59	1·07	18·8	0·067	15·9
19–36	..	..	152	..	5	..	(154)
0–36	..	..	256	..	36	..	(36·1)
Experiment B. 22.iii to 17.iv.35. *Lauderia borealis* Gran.							
0–7	0·642	+3·0	85	9·42	12	0·264	35·7
7–11	1·000	−2·6	11·6	0·828	22·5	0·317	2·61
11–17	0·540	−0·19	5·33	0·705	14	0·367	1·92
17–21	0·040	+0·2	4·5	..	−0·5	..	..
21–26	(decrease)	+0·85	1·0	..	−0·5	..	..
0–17	2·182	..	98·5	3·38	48·5	0·313	10·8
0–26	..	..	104	..	47·5	..	11·9
Experiment C. 29.iii to 17.iv.35. *Lauderia borealis* Gran.							
0–4	0·195	−1·1	8	2·93	2·6	0·187	15·6
4–10	0·684	−2·4	69	7·21	16	0·33	21·8
10–14	0·680	−0·35	38	4·0	13	0·27	14·9
14–19	..	+1·75	−0·9	..	−0·1	..	..
0–14	1·56	..	115	5·26	31·5	0·285	18·5
0–19	..	..	114	..	31·4	..	18·4
Experiment D. 29.iii to 17.iv.35. *Chætoceros decipiens* Cleve.							
0–4	0·367	−0·06	2	0·390	11	0·422	0·92
4–10	0·751	−4·0	86	8·21	14	0·276	29·8
10–14	0·240	+0·2	30	8·93	8	0·465	19·2
14–19	(decrease)	+1·5	3·6	..	1·6	..	12·5
0–14	1·36	..	118	6·20	33	0·342	18·2
0–19	..	..	121·6	..	34·6	..	17·6

γ = millionths of a gramme; ∴ γ/litre = mg./m³.
γ-atoms = γ divided by the atomic weight of the element in question (see Cooper, 1933*d*).

discussed later when we come to consider the nitrogen cycle in detail. For the present the important point is the connection which appears to exist between its appearance and the death of plankton organisms.

In those of the culture experiments which were continued long enough (*e.g.* Experiment A, Table II, and Text-fig. 1), the nitrite, after its sharp rise, showed a rapid fall. This


γ/litre
NO3-N
P2O5
NO2-N X10
300. 60.
250. 50.
200. 40.
150. 30.
100. 20.
50. 10.
0. 0.
(11·5)
NO2
NO3
P2O5
Cells
Cells/c.c.
4000
3000
2000
1000
0
0
4
11
19
25
32
39
DAYS
Experiment A. 14 Feb to 22 Mar 1935. Chaetoceros.
γ/litre
NO3-N
P2O5
NO2-N X10
300. 60.
250. 50.
200. 40.
150. 30.
100. 20.
50. 10.
0 0
Cells/cc
2500
2000
1500
1000
500
0
0
7
11
17
21
26
DAYS
Experiment B. 22 Mar to 17 Apr 1935 Lauderia.

TEXT-FIG. 1.

corresponds well with the very sharp peaks in the nitrite profiles observed in the sea, and suggests that we are dealing with the same phenomenon.

IV. THE RESULTS OF THE JOHN MURRAY EXPEDITION.

(i) The Interpretation of Chemical Results.

Before going on to discuss the results of the investigations carried out at sea, it will be necessary to consider a few points involved in their interpretation. The importance of chemical investigations as an aid to the understanding of an observed distribution of phytoplankton has been emphasized by Brandt, and many of the conclusions drawn from his and subsequent work have been summarized in Section II. The value of the combination has been well illustrated by Braarud and Klem's (1931) investigations off the coast of Norway, by Moberg's (1928) paper on the effect of upward water movements, and by Hentschel and Wattenberg's (1930) work in the South Atlantic. An attempt has, however, been made in the following pages to make an even fuller use of the knowledge gained not only at sea, but from cultural experiments, and for this purpose further discussion of certain aspects of the problem is necessary. It should be emphasized that the inferences here drawn from the chemical data are largely theoretical, and are as yet unconfirmed by direct observation.

(*a*) *Oxygen.*

The depth at which the oxygen produced by photosynthesis just balances that consumed by respiration has been called by Marshall and Orr (1928) the " compensation point ". This depth will clearly vary from moment to moment with the varying amount of light reaching the water surface and penetrating the water, but for our purpose the important level is the balance level for the 24 hours, since this must mark the limit below which light is insufficient for continued growth of the phytoplankton. Gran found it in Woods Hole Harbour in August, 1932, at about 6 metres, when simultaneous measurements by Clarke and Oster (1934) of the penetration of light show that the illumination at this depth was approximately 1·5% of that at the surface. The sea on this occasion was calm and the sky clear.

Now, although the compensation point is not the depth at which the illumination is reduced to a particular fraction of that at the surface, but rather the depth at which it is reduced to a particular absolute value,* yet in dealing with the clear skies and uniform conditions of the Arabian Sea during the NE monsoon, we may take as an indication of the depth of the compensation point that depth at which the light is reduced to about 1·5% of its surface value.

Poole and Atkins (1929) have shown that we may use the Secchi disc reading as a measure of the opacity of the water according to the relation—

$$\dagger\mu = \frac{1{\cdot}7}{D} \ (D = \text{the Secchi disc reading in metres}).$$

* Pettersson, Hoglund and Landberg (1934) found this value to be about 400 lux for mixed plankton.

† This quantity has various names and symbols in the literature. Atkins also calls it both λ and μ_v at different times. It has the names " absorption co-efficient ", " transmissive exponent " (Clarke and Oster) and " extinction co-efficient " (Pettersson).

Now the illumination at depth d metres is obtained from the extinction co-efficient by the relation—

$$\mu_{o-d} = \frac{\log_e 10}{d} [\log (100 - s) - \log p_d],$$

where μ_{o-d} is the extinction coefficient of the water layer $0 - d$ metres,
s is the percentage of the incident light lost by reflection at the surface ;
p_d is the percentage of the incident light which penetrates to the depth d.

It therefore follows that the depth at which the illumination is reduced to 1·5% of that at the surface is given by—

$$d = \frac{D \log_e 10}{1{\cdot}7} [\log (100 - s) - \log 1{\cdot}5].$$

In the very calm conditions usually prevailing, and with the sun at a considerably higher altitude, Poole and Atkins's average figure of 15% for s, the surface loss, is certainly too high for the Indian Ocean at the time of the expedition. They record a figure as low as 5% for one occasion in the English Channel. If we take the figure 5%, we obtain from the equation above—

$$d = 2{\cdot}44\ D.$$

In the absence of any data for surface loss or for surface illumination, we cannot determine the compensation level with any accuracy, but the value $2{\cdot}5 \times D$ will give us an approximate idea of the depth limit, beyond which photosynthesis is unlikely to balance the respiration of the phytoplankton.*

If we started with homogeneous water 100% saturated with oxygen, and allowed plankton to grow in it, we should find after a time that the water below the compensation level lost oxygen, while that above it became supersaturated. In the sea, however, such ideal conditions are very seldom realized. Since the water is never 100% saturated when plankton production begins, and because there is always zooplankton present, the depth at which the water is just saturated can only very rarely be taken as indicating the compensation level.

In practice, then, we shall find that in the Indian Ocean the water at the compensation level, as indicated by the Secchi disc reading, is fairly constantly about 85–90% saturated, where there is a sufficiently thick layer of homogeneous surface water. Where the oxygen falls much below this value, it is safe to infer that conditions are not in equilibrium so far as concerns the relation between the plankton and light, often owing to some disturbing factor such as a sharp discontinuity layer. Such conditions occur for example in some of the Gulf of Aden stations (see Text-fig. 2). It must, however, be remembered that exact agreement is not always to be expected, since the determination of the compensation level from the Secchi disc reading is only an approximation. Thus Atkins's relation, quoted above, between the Secchi disc reading and the extinction coefficient is only an approximate one, and, secondly, the calculation involves the assumption that the transparency of the water below the point at which the disc disappeared is similar to that of the water above—an assumption which is not always justified.

* Miss P. M. Jenkin tells me that in her experiments on the growth of diatom cultures in the sea (not yet published) she finds the compensation depth to be about 2·5 to 3 times the Secchi disc reading. It should be noted that most of her work was done at Plymouth in the long calm days of summer, when the compensation level for a given illumination is probably deeper than it would be in the tropics.

It will be observed that in a great number of the John Murray Expedition stations the Secchi disc-compensation level coincides with the lower limit of the layer of water depleted of nutrient salts—a good indication of its general validity. In hardly any cases does it fall above that limit ; where it does, the reason may be either a general downward movement of the water, or a lack of uniformity in the transparency of the water, owing, for example, to a fresh plankton outburst in the upper levels.

The oxygen profile (% saturation plotted against depth) can tell us a great deal about conditions at any given station, especially when considered in conjunction with other data. Owing to the presence of phytoplankton, and to the possibility of direct gaseous exchange with the air, the surface layers are never very far from saturated with oxygen, unless they consist of water which has recently come up from the dark depths. But at a few metres below the surface, unless there is strong mixing by wind, the oxygen content depends on the balance between production by phytoplankton, and consumption by zooplankton and bacteria. A high oxygen will therefore indicate vigorous production of phytoplankton, and this may lead to a high degree of supersaturation during the enormous spring outburst of the temperate and polar regions (*e. g.* Gaarder and Gran, 1927, etc.). Supersaturation is not very common in tropical regions (apart from peculiar localities such as the shallow water over coral reefs), but may be seen occasionally (*e. g.* Stations 31 and 173.* The oxygen curve frequently shows a maximum value well below the surface. This is due to the fact that the light is often too intense at the surface (Marshall and Orr, 1928), so that the zone of maximum productivity lies deeper. The plankton diagrams (*e. g.* Stations 130, 132, 167, 169,† Text-figs. 12 and 13) show that where such a peak is present, it usually occurs at a level just above that of the plankton maximum. Exactly the same relation can be seen in the recorded results of other workers, *e. g.* Gran (1929*b*, 1930, 1933), Gran and Thompson (1930), Gaarder and Gran (1927), Braarud and Klem (1931), Braarud (1934), so that we may conclude that it is commonly the case.

As to the reason for this we have no conclusive evidence, but there are several possible contributory factors. In the first place all the phytoplankton organisms enumerated by counting, or estimated by their pigment content, are not necessarily in an actively growing condition. This is borne out by the fact that it is not by any means every diatom picked out from the plankton which will grow in culture. It was repeatedly observed that when a single cell or a very small number of cells of a species was found in a tow-netting, no culture could be obtained of that species, while a species which was abundant in the plankton would nearly always give vigorous cultures. It follows that the total population is not necessarily a true measure of the productivity, if we define productivity as the rate of carbon assimilation and cell increase.

Now it has been repeatedly observed (Marshall and Orr, 1927 ; Gran, *passim* ; Føyn, 1929) that after a period of activity diatoms and other phytoplankton stop growing and sink. This can also be observed in the laboratory, for in a young culture the cells remain well in suspension in the medium, whereas when the culture is several days old and contains

* It may in fact occur more often than it is recorded, since the fact of supersaturation greatly increases the difficulty of avoiding loss of oxygen between the time of collecting the sample and the time of adding the Winkler reagents. This difficulty is considerable in the high air temperatures of the tropical day.

† Here although the maximum recorded is for the 0–40 m. layer, this was probably due to a very dense population at the bottom of this layer.

a dense population, the cells sink to the bottom of the flask, and little, if any, further growth occurs. This happens although the cells may be still healthy and normal in appearance, and will give further good growth on subculturing. In the sea, then, the population at any level in the illuminated zone consists partly of cells which are growing and dividing, and partly of cells which are senescent and merely sinking, and as we go downwards the proportion of growing cells will decrease, as will their actual numbers. The senescent cells, on the other hand, will not only increase in proportion to the growing ones, but also in total numbers, until the compensation point is reached. We shall therefore expect to find the densest population below the level at which multiplication is most rapid to an extent which will depend on the rate of sinking. Furthermore, any change in the density gradient of the water itself will affect the observed phytoplankton distribution through its effect on the rate of sinking of the dead and dying organisms; in particular a rise will tend to cause artificial plankton maxima. To sum up, then, the phytoplankton maximum may not necessarily be taken as indicating the level of maximum productivity, which is more accurately given by the oxygen saturation curve. In water where the density gradient is approximately uniform and fairly small, the plankton maximum will be expected to occur somewhat below the oxygen maximum.

Since the oxygen produced by photosynthetic organisms is merely a by-product of carbon assimilation, its production is always associated with the consumption of the nutrient salts (especially nitrate and phosphate) which are also necessary for the construction of the plant body. It is therefore to be expected that in stable conditions, where the composition of the sea-water is largely determined by the activities of the plankton, the profiles of oxygen and nitrate content will be mirror images. In a great number of cases this relation is found to hold with remarkable accuracy, as may be seen from the diagrams (*cf.* also Redfield, 1934). Where the relation does not hold we may infer the operation of disturbing factors, and comparison with other chemical properties of the water makes possible various deductions about the conditions. Thus high oxygen may accompany comparatively high nutrient salt concentrations. This indicates the early stages of plankton production, when considerable amounts of oxygen have already been liberated, but the limit set by the supply of nutrients has not yet been reached. Such conditions are commonly found at the beginning of the spring outburst in temperate waters, and may be seen at Stations 136, 166 and 167, where it is therefore inferred that there has been a recent influx of unexhausted water from elsewhere. Conversely, the zone of more than 85% saturation may not extend quite down to the limit of the layer which has been depleted of nutrients. This is rather rare, and the depth discrepancy is never great, but it was found at Stations 32, 76, 92, 173 and 182. The depletion of nutrient salts shows that there had been a good growth of phytoplankton, but this had ceased, and given place to an abundant zooplankton, or more probably provided nutriment for a rich bacterial flora, which had consumed much of the oxygen.

(*b*) *Nitrite.*

Nearly all the stations show the presence at the lower limit of the nutrient-depleted surface layer of a narrow zone of water which has a high nitrite content, ranging up to about 20 mg. N/m^3. Where, as is very often the case, this peak nitrite value also falls at a density boundary, it is even more pronounced than otherwise.

Similar accumulation of nitrite in the thermocline has been observed elsewhere—

e. g. Moberg and Fleming (1933) off Southern California, Robinson and Wirth (1934*b*) off Vancouver Island, Braarud and Klem (1931) off the Norwegian coast, Orr (1926) in the Clyde area. Soot-Ryen (1932) off the Norwegian coast found that nitrite maxima appear in the upper layers (0–100 m.) about the times of the spring and autumn plankton maxima, in each case moving down later to the bottom. This appears to be associated with a sinking mass of dying plankton. Verjbinskaya's (1932) results show that in the Barents Sea a large production of nitrite similarly accompanies plankton production, reaching high values at depths of 50–100 metres, especially at boundaries such as the bottom of the Arctic water. In winter, when there is little plankton and much mixing, no nitrite is present.

Cooper (1933*a*) did not find a similar depth distribution of nitrite in the English Channel, perhaps owing to the fact that there is no appreciable discontinuity layer at the time of the spring plankton maximum. In a later paper, however, he (Cooper, 1933*b*) gives figures for station E 1 in August, 1932, when the water was very stable, and there we do find a small peak in the nitrite profile at the bottom of the thermocline.

Deacon (1933) finds in the Antarctic water up to 8 mg. NO_2–N/m^3 in the surface 100 metres, but never any below the discontinuity. In the sub-antarctic zone he finds up to 30 mg. N/m^3 just below the discontinuity layer which marks the bottom of the tropical water.

As far as it goes, previously recorded data seem to show a similar distribution to that found in the Indian Ocean region. Excepting Orr's (1926) paper, the phenomenon seems to have attracted little attention, and most authors have either made no comment, or passed it over with a brief remark to the effect that nitrite is a stage in the nitrification of organic residues—a process about which little is known. Unfortunately in spite of a good deal of scattered information about bacteria, we still do not know very much about the part they play or about the nitrification process,* but in view of the considerable amount of data accumulated on the John Murray Expedition, we are in a position to generalize to some extent on the distribution of nitrite, and its relation to that of the phytoplankton.

From the presence of relatively large quantities of nitrite just below the zone of maximum productivity, at levels where we should expect to find dead and dying phytoplankton sinking towards the depths, it seems probable that the two are connected. This is supported by the fact that these zones of high nitrite are especially prominent where a rapid change in the density of the water tends to hold up the sinking particles,† and further by the cultural experiments already described (*cf.* also Orr, 1926, where he mentions that nitrite increased in cultures, continuing to increase after the diatoms had ceased to do so). The quantity of nitrite found depends on the relative rates of formation and destruction, and does not bear any direct quantitative relation to the amount of plankton material. However, since its formation must depend on the supply of organic matter,‡ it is a reasonable inference that a high nitrite concentration indicates the production of rich supplies of organic material by the plankton, while a low nitrite may have several explanations. In conjunction with abundant plankton it indicates that

* See below, p. 62.

† That a discontinuity does tend to cause an accumulation of particulate matter is clearly shown by measurements of the penetration of light, *e. g.* Oster and Clarke (1934), Uterback (1933), and Pettersson (1935).

‡ See below, p. 65.

production has recently begun, so that there is as yet no great amount of organic matter free in the water—the initial phases of an outburst; such conditions will usually be accompanied by relatively high concentrations of nitrate and phosphate in the illuminated layers. This stage is a very short one, and a certain amount of nitrite can be formed before all the nitrate and phosphate are exhausted. Where plankton is sparse, this is in itself an explanation, and the absence of nitrite indicates that it has been sparse for some time. Such conditions are most commonly found in temperate and polar seas in winter, when lack of light limits plankton production. Where the discontinuity layer is absent, or near the surface, so that the productive zone extends well below it, there will be no tendency for an accumulation of organisms at any one level, and there may be no level at which the production of nitrite greatly exceeds its destruction, and therefore no level at which any large amount is found. Under these conditions the absence of a marked nitrite peak does not necessarily indicate a small plankton production.

(ii) The Distribution of Nitrogen Compounds in the Northern Indian Ocean.

The John Murray Expedition was at work for nine months from September, 1933, to May, 1934. The area investigated, and the positions of the stations at which nitrate, nitrite or phytoplankton determinations were made in addition to the routine observations of temperature, salinity and oxygen are shown on the chart by their serial numbers in the expedition's station list.

The results of the analyses are shown graphically in Text-figs. 2 to 13, which represent profiles of the upper 300 metres of the water column, concentration being plotted as abscissa against depth as ordinate. The full data are also given in the tables in the appendix to Vol. II of these reports. Concentrations of nitrate and nitrite are given in milligrams of nitrogen per cubic metre of water (mg. N/m^3); the oxygen content is given as the percentage of the saturation value for water of similar salinity and temperature; the pH values given have been corrected for salinity and temperature, and show the pH of the water *in situ*. In the stability diagrams the width of the figure is proportional to the average rate of change of density (σ^t units per 10 m. depth) between any two sampled depths—negative values are shown white. It should be noted that in these calculations the effect of pressure has been neglected. This is not strictly permissible, and does not give the true value for the stability, but at the small depths here studied the results are quite sufficiently accurate for the purposes of this discussion. The phytoplankton is reported in arbitrary units of plant pigment per cubic metre of water; in order that the figure might be comparable with his for the English Channel, the unit devised by Harvey (1934*a*) was used. Each rectangle in the diagrams shows on the horizontal scale the average plankton content, and on the vertical scale the portion of the water column fished by the net on each haul.

The Secchi disc readings are shown by a short horizontal line near the right-hand edge of the figure, the depth being given below it thus: $\overline{\text{SD} = 22{\cdot}5}$ m. The horizontal broken line across the figure shows the compensation depth calculated as $2{\cdot}5 \times$ the SD reading (see p. 37).

In the discussion of the results which follows, the stations are considered in groups, each of which represents a section across a body of water. In some cases diagrams to

show the distribution of nitrate in the section are given. The movements of the water have been deduced partly from these diagrams. These movements have been confirmed and amplified by information kindly supplied by Dr. E. F. Thompson, and derived by

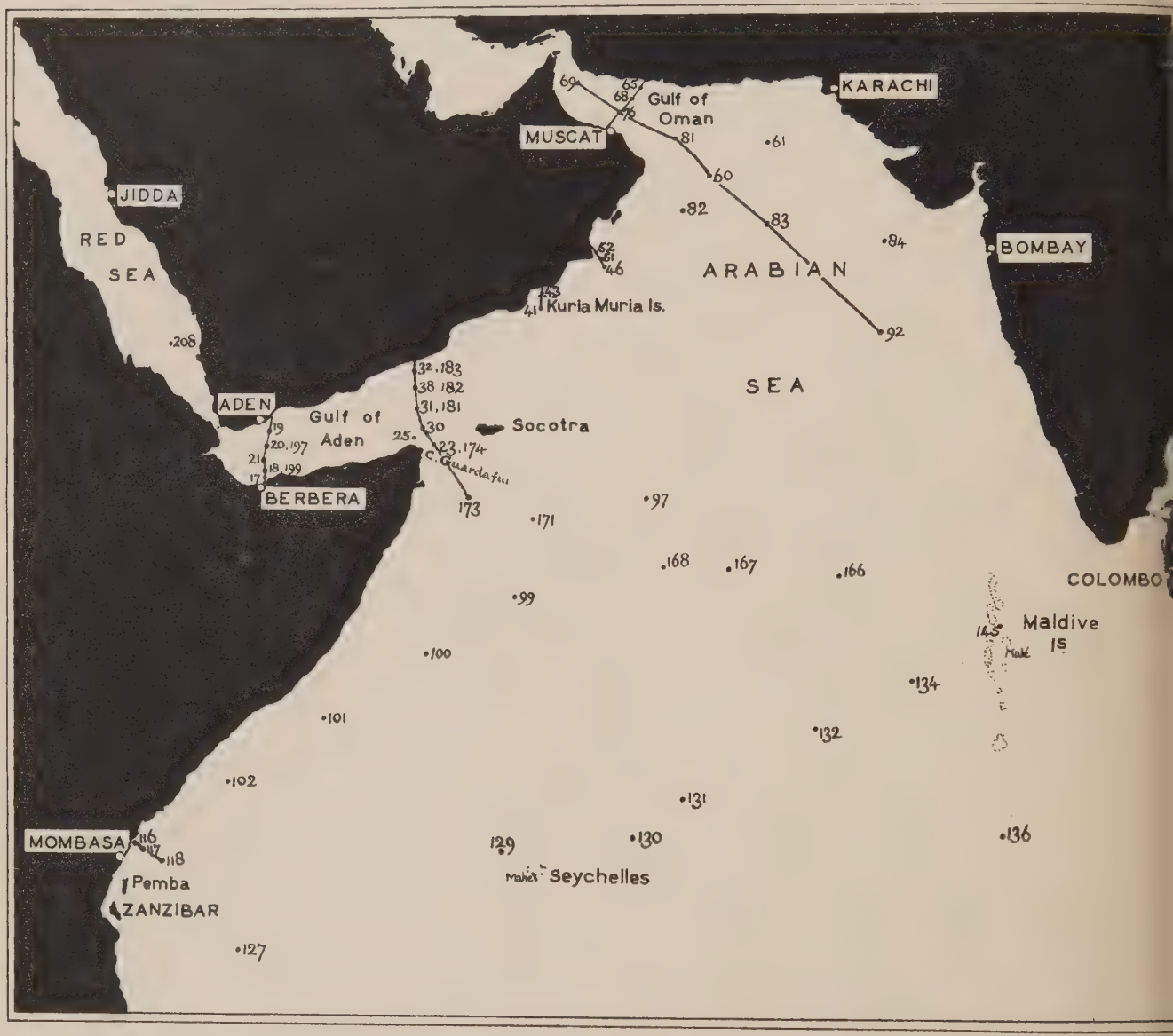


Chart.—The area investigated and stations where determinations were made.

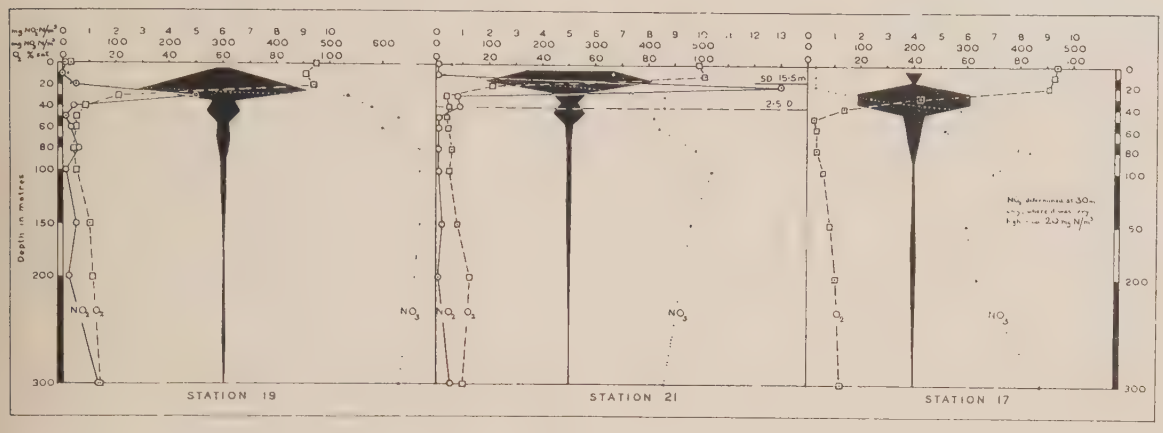
him from his work on the salinity and temperature data ; this applies especially to the questions of the origin of the various masses of water.

(1) *The Gulf of Aden.*

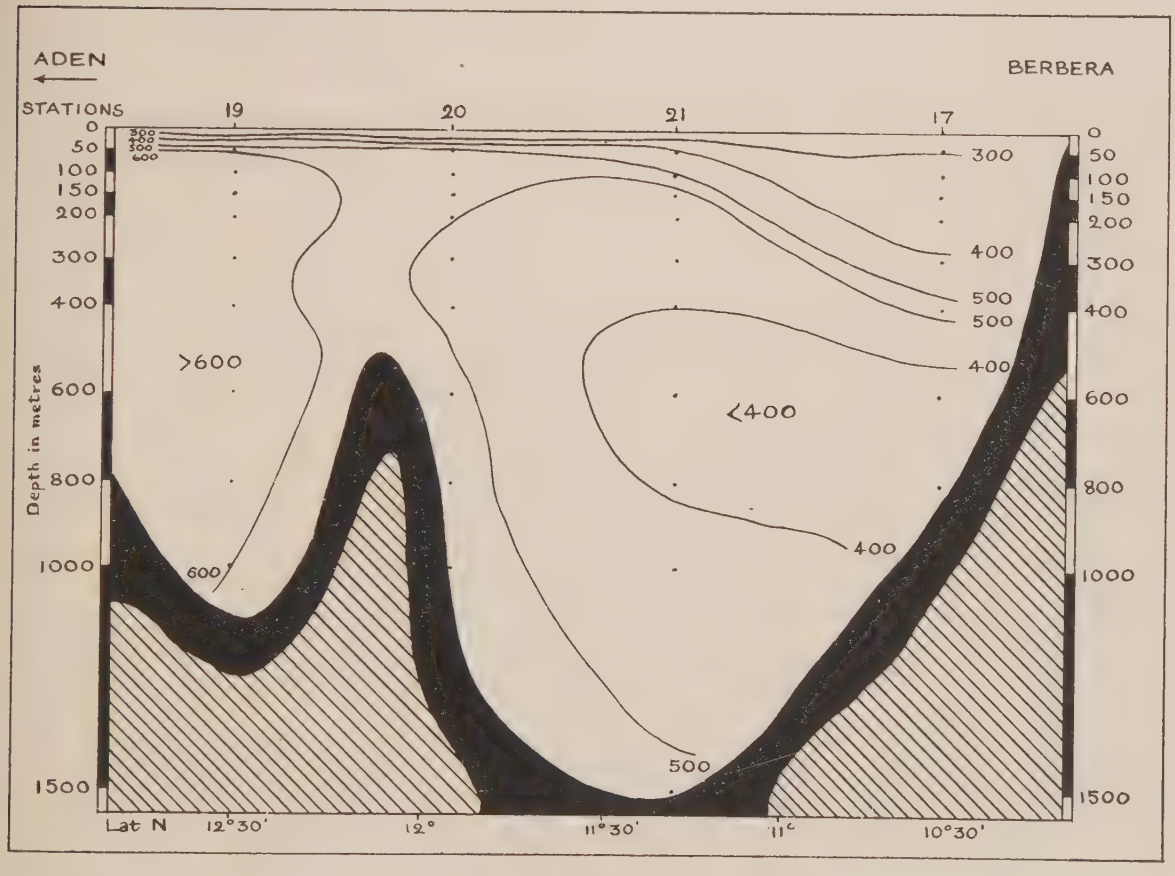
Aden Section. Stations 17–21. September, 1933. (Text-figs. 2 and 3.)

The distribution throughout this section conforms closely to a single type dominated by the presence of a very sharp discontinuity layer (see Text-fig. 2). In the northern

half of the section this lay between 20 and 30 metres; at Station 21 upwelling of water from below had pushed it up into the 10–20 metre layer. There was a sharp division at



TEXT-FIG. 2.



TEXT-FIG. 3.

this level between the warm, saline, surface water, which was depleted of nitrate and approached saturation with oxygen, and a lower layer of cooler, less saline water with a

very low oxygen content. Nitrite reached high values just below this density boundary, owing to the decay of sinking plankton held up at that level.

The deeper layers show a more or less uniformly high nitrate concentration, tending to be higher in the northern half of the section. This is readily explained by the section diagram (Text-fig. 3), which shows that there was a mass of water rich in nitrate (more than 500 mg. N/m^3) hugging the northern shore. This water comes originally from the depths of the southern part of the Indian Ocean. It is carried north in a current which sweeps along the coast of Africa, rising nearer the surface on its way, until finally it strikes the Arabian coast. It then divides, and part moves west along the northern shore of the Gulf of Aden. On the south side of the Gulf was a mass of water lying between 400 and 1000 metres, which contained less than 450 mg. NO_3–N/m^3, and had a very high salinity and temperature. This is mid- and bottom-water from the Red Sea moving east down the Gulf towards the open ocean.

Near the southern shore the downward trend of the iso-nitrate lines shows that the wind had piled up the water of the top 200 metres against the coast, causing a downward movement of surface water.

Gardafui Section. Stations 23–38. October, 1933. (Text-figs. 4 and 5.)

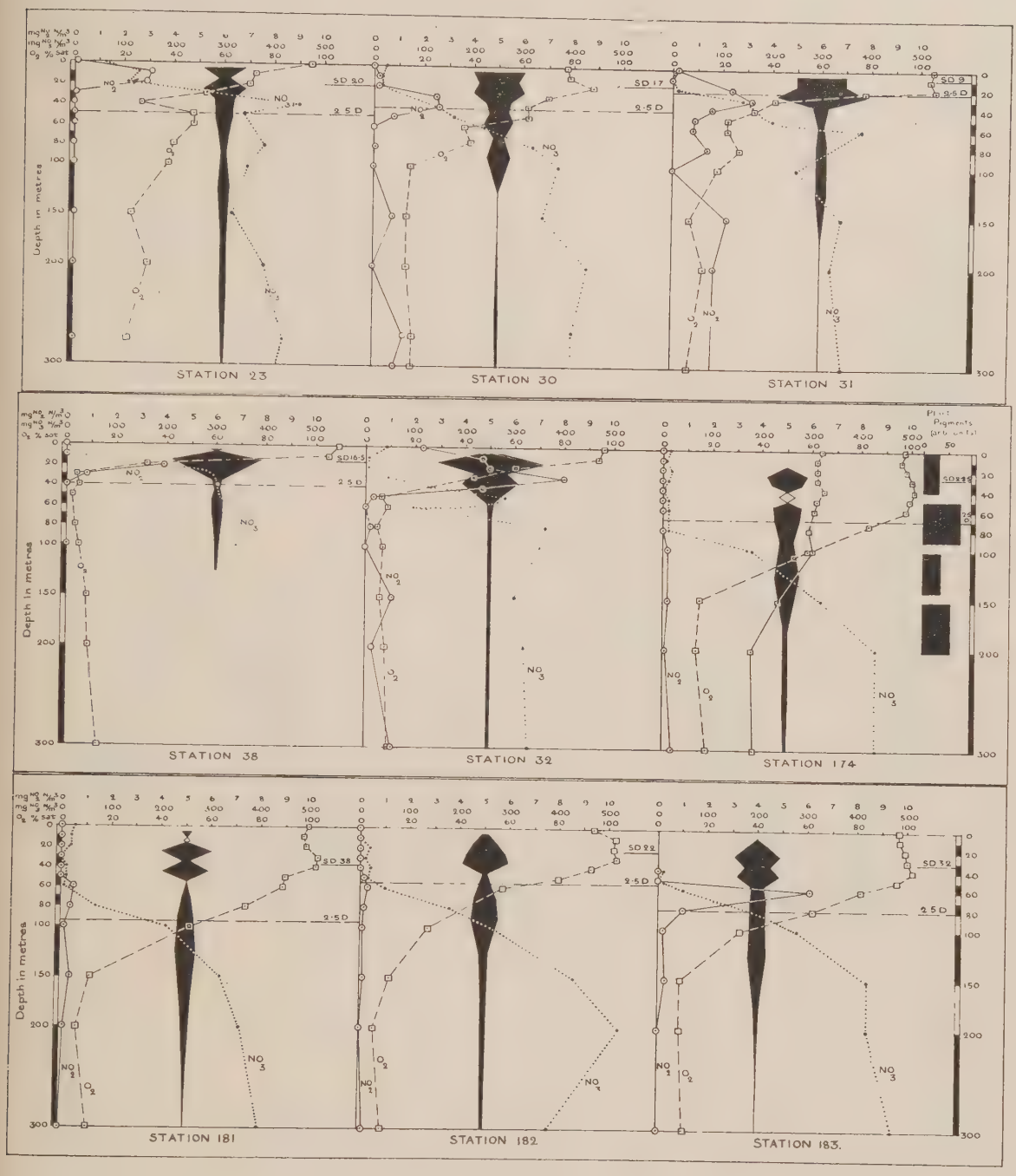
The main water masses that we saw in the more westerly section in the longitude of Aden were here in somewhat different positions (see Text-fig. 5). The oceanic water containing more than 450 mg. N/m^3 was here found entering the Gulf at the bottom near the south side, where it was pushed up towards the surface by the ridge which runs between Cape Gardafui and the Island of Socotra. The Red Sea water had mostly moved over to the northern shore, where it forms a vortex in the middle depths. A small proportion moves further south and out into the open ocean, but as it also moves to a higher level among water of a similar nitrate concentration, it does not appear on the nitrate diagram. The result of the upward movement over the Socotra-Gardafui ridge is to bunch together the layers below the surface, so that the water found at Station 23 at about 100 metres was similar to that found about the 300-metre level at the north end of the section. The effects of this turbulence are clearly seen at Station 23 (Text-fig. 4), where the stability was comparatively low, and nitrate was high and oxygen low up to 10 metres below the surface.

As we go north along the section away from this area of turbulence, the stability increases, and we pass gradually into conditions more like those at the western end of the Gulf. At Station 38 we again find the sub-surface layer bunched together somewhat, but there was not the same degree of turbulence as at Station 23. Nitrite again showed peaks in the boundary layers.

Gardafui Section. Stations 174–183. May, 1934. (Text-figs. 4 and 5.)

The deep ocean water which, in October, was moving into the Gulf on the south side of the section between 600 m. and the bottom, now filled the entire bottom of the Gulf, as it poured over the Socotra–Gardafui ridge. The outgoing water from the Red Sea had been pushed upwards, and now occupied a central position between 300 and 600 metres (see Text-fig. 5).

Along the Arabian coast the change from the strong winds of the SW monsoon season to the gentle off-shore winds of the NE monsoon had produced considerable

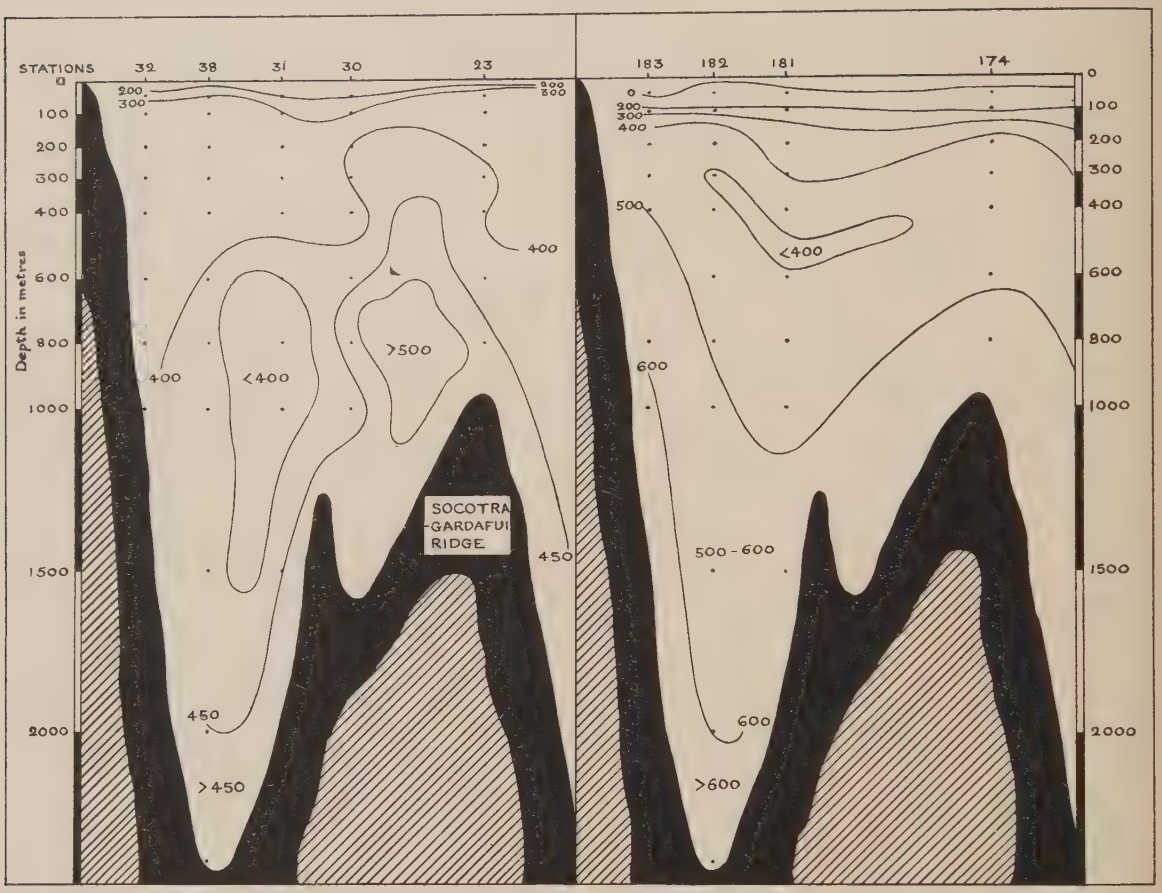


TEXT-FIG. 4.

changes. There was no longer water piled up against the coast and so forced to sink, as at Station 32. Instead there was at Station 183 a tendency for the deeper water to approach the surface, and this tendency was even more marked at Station 182. Water

was also being pushed up at Station 174, as the deep water poured over the Socotra-Gardafui ridge.

The calm weather had allowed a much thicker warmed layer to develop at the surface, although with increasing thickness of this layer the temperature gradient had become less steep, and the stability less than it was in October (see Text-fig. 4). The higher transparency indicates that the plankton had either diminished, or sunk to lower levels, and probably the latter is principally the case. The oxygen curves all show peaks at about



Text-fig. 5.

30–40 metres, which was therefore the zone of maximum production. This is borne out by the plankton data of Station 174, where the richest haul was obtained between 50 and 90 m.*

Nitrite curves show small peaks just below the limit of the layer depleted of nitrate. Where this falls at a boundary, the peak is more pronounced (*e. g.* Station 183).

In October the moderate oxygen concentrations in the surface layers, the high nitrite figures, and the depletion of nitrate in the upper 20 metres, indicate that phytoplankton had already passed its zenith, and was limited by lack of nutrient salts in the productive zone. Although the very stable conditions were partly due to the different origins of the water, they were probably of comparatively short standing, and were changing rapidly

* This statement appears paradoxical, but see p. 38.

as the result of the recent change from rough to calm weather. The depth of the layer depleted of nitrate falls in most of the stations far short of the calculated compensation point, although the transparency was low. This is probably because the sharp discontinuity was holding up the growing organisms, and preventing their colonizing the unexhausted water below, so that conditions were not in a static equilibrium. It is also possible that any which did penetrate would develop much more slowly in the cooler water below.*

Unfortunately no quantitative plankton observations were made, but the area was observed to be rich in the larger zooplankton. The bulk of the surface plankton caught in the high-speed tow-net was species of *Ceratium* with a few Copepods and *Sagitta*. This is the type of plankton to be expected in warm water poor in nutrients, and Col. Sewell suggests that it was probably brought in by the surface East African coastal current.

Since there are no rivers in the area, and almost no rainfall, the low transparency cannot be attributed to material washed out from the surface of the land. Further, since the water is mostly deep, and the shores are steep-to and rocky, it is not easy to see how much detritus can have been stirred up off the bottom, even by the storms of the SW monsoon. The suspended matter in the sub-surface layers must, then, have been mainly zooplankton organisms—a not improbable conclusion, since the area, as one of considerable vertical mixing, would be expected to be a productive one.

In May conditions were more nearly in equilibrium. Warming by the sun had pushed the thermocline deeper, and the plankton penetrating through the less sharp discontinuity had carried the depletion of nitrate almost to the limit set by illumination.

Owing to supplies of nutrients brought up by turbulence, from which the plankton was no longer cut off by a steep and shallow thermocline, there was active production in progress, maintaining high oxygen concentrations. The low nitrite figures are a little surprising, since in view of the extensive depletion of nitrate, production cannot be supposed to have begun very recently.† The reason is perhaps the absence of any marked discontinuity at the lower limit of the productive zone, so that there might be no tendency for an accumulation at any one level. It must also be remembered that the layers of high nitrite are often very thin and sharply defined. It is therefore possible that, especially in the calm conditions prevailing with little mixing by wind, observations 10 metres apart might miss the maximum value, so that the absence of a peak in the recorded curves does not necessarily show its absence in fact. Since conditions in the surface layers were very uniform throughout the section as a whole, it may be that whereas in October the depths sampled tended to strike the peak value at all stations, in May they similarly tended to miss it at all stations.

(2) *The Arabian Coast.*

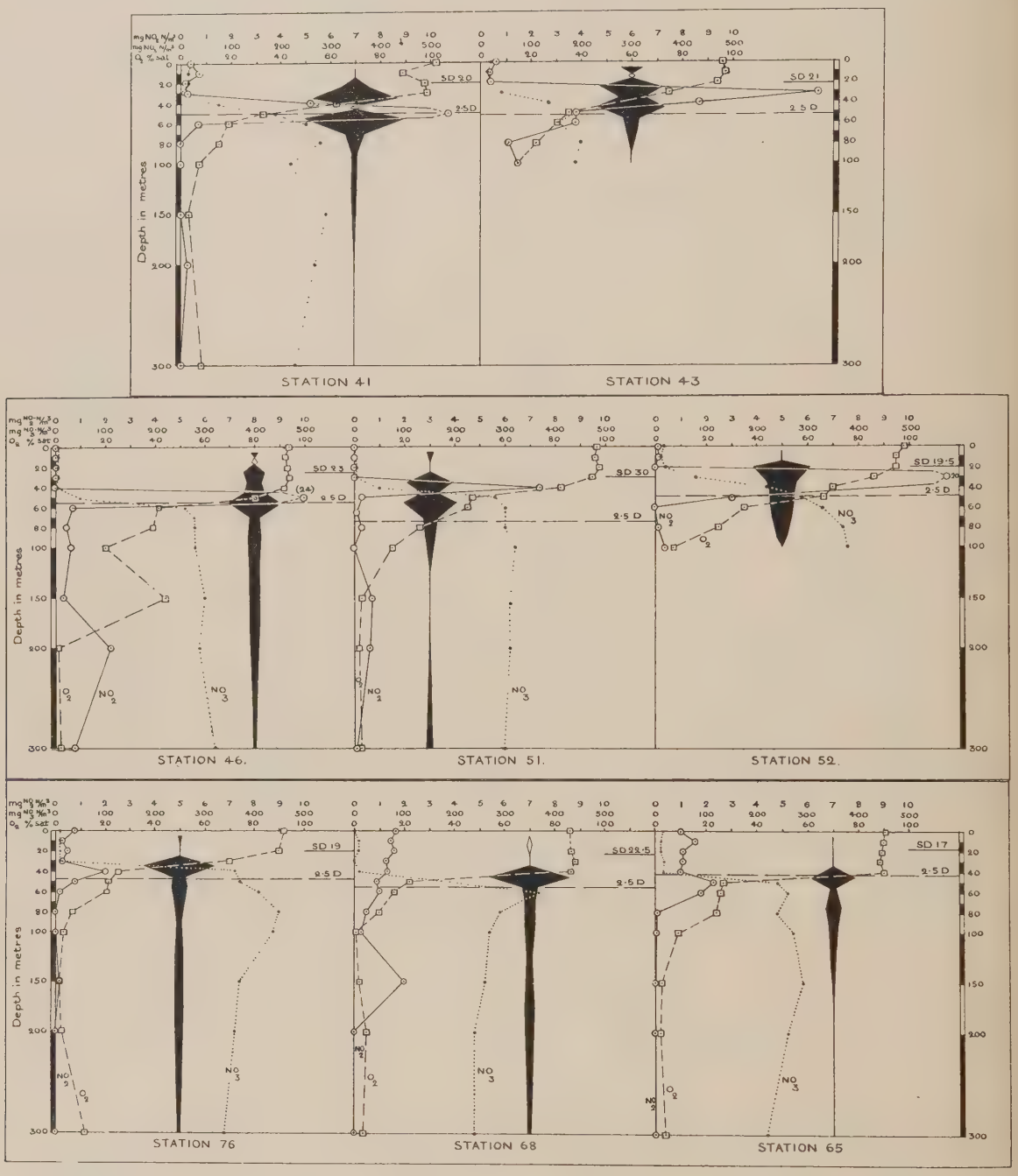
Arabian Coast Sections. Stations 41–52. October, 1933. (Text-figs. 6 and 7.)

Conditions here appear to have been in the transition phase between the two monsoons. The upwelling along the coast, which occurs in the SW monsoon season, was falling off,

* For similar limitation of the productive layer by the thermocline, see Seiwell (1935), who attributes the effect to the influence of temperature on the organisms.

† The May outburst recorded by Hornell and Ramaswami Nayudu (1923) off the Malabar coast is definitely correlated with the rain brought by the SW monsoon or the unsettled weather which precedes it. Similar conditions do not obtain here, where the monsoon brings little or no rain, and in any case had not yet broken at the time under discussion.

and only appears in the water below 100 metres and some way off shore. There was, however, a slight upward movement in the surface layers at Station 52, probably owing to the NW wind blowing at the time of observation. As a result of this movement it

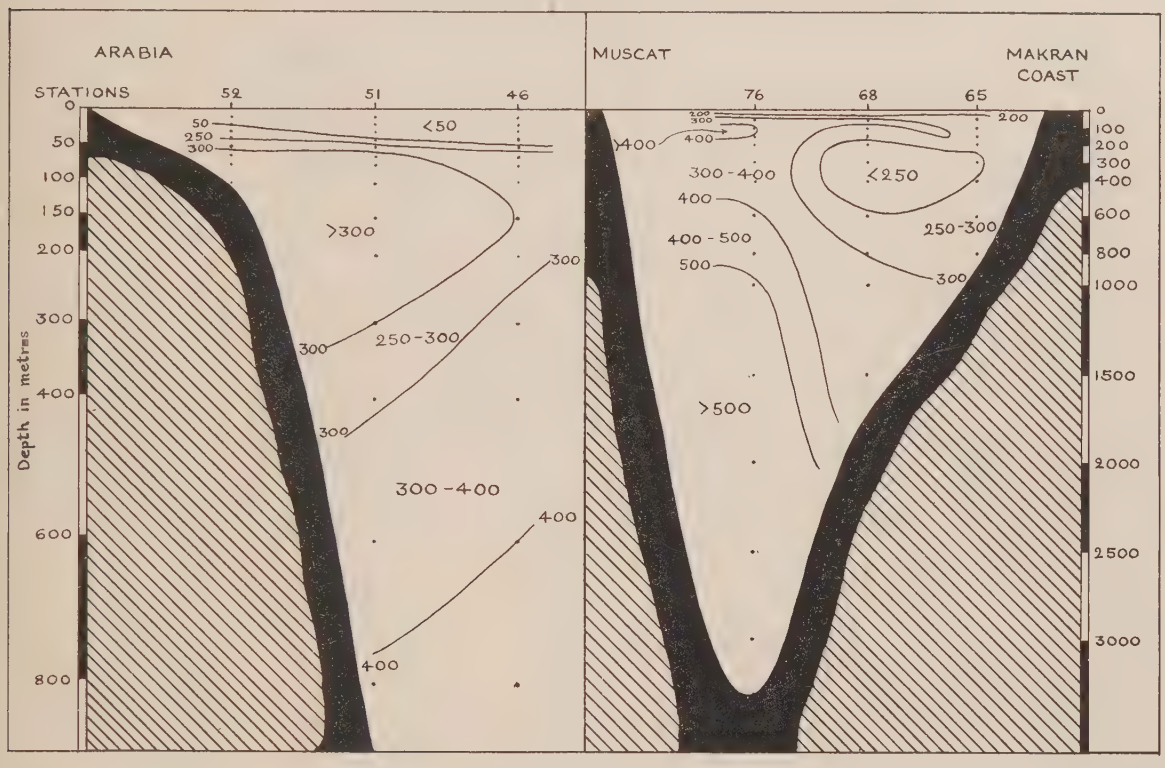


TEXT-FIG. 6.

was only the well-mixed surface layer which was depleted of nutrient salts at the inshore stations, while in the more stable conditions further out at Station 46 depletion had proceeded down to 40 metres.

The oxygen, nitrite and transparency figures suggest that a period of considerable

plankton production was just coming to an end, and there were great quantities dying off in the discontinuity layer. This is confirmed by the abundant zooplankton, especially salps, which was observed here. As far as plankton is concerned, the area resembles the Gulf of Aden. The supplies of nutrients brought up from below by the SW monsoon support a large but short-lived outburst of phytoplankton, which develops in the increasingly stable conditions following the change of wind, and subsequently provides food for a rich zooplankton.



TEXT-FIG. 7.

(3) *The Gulf of Oman.*

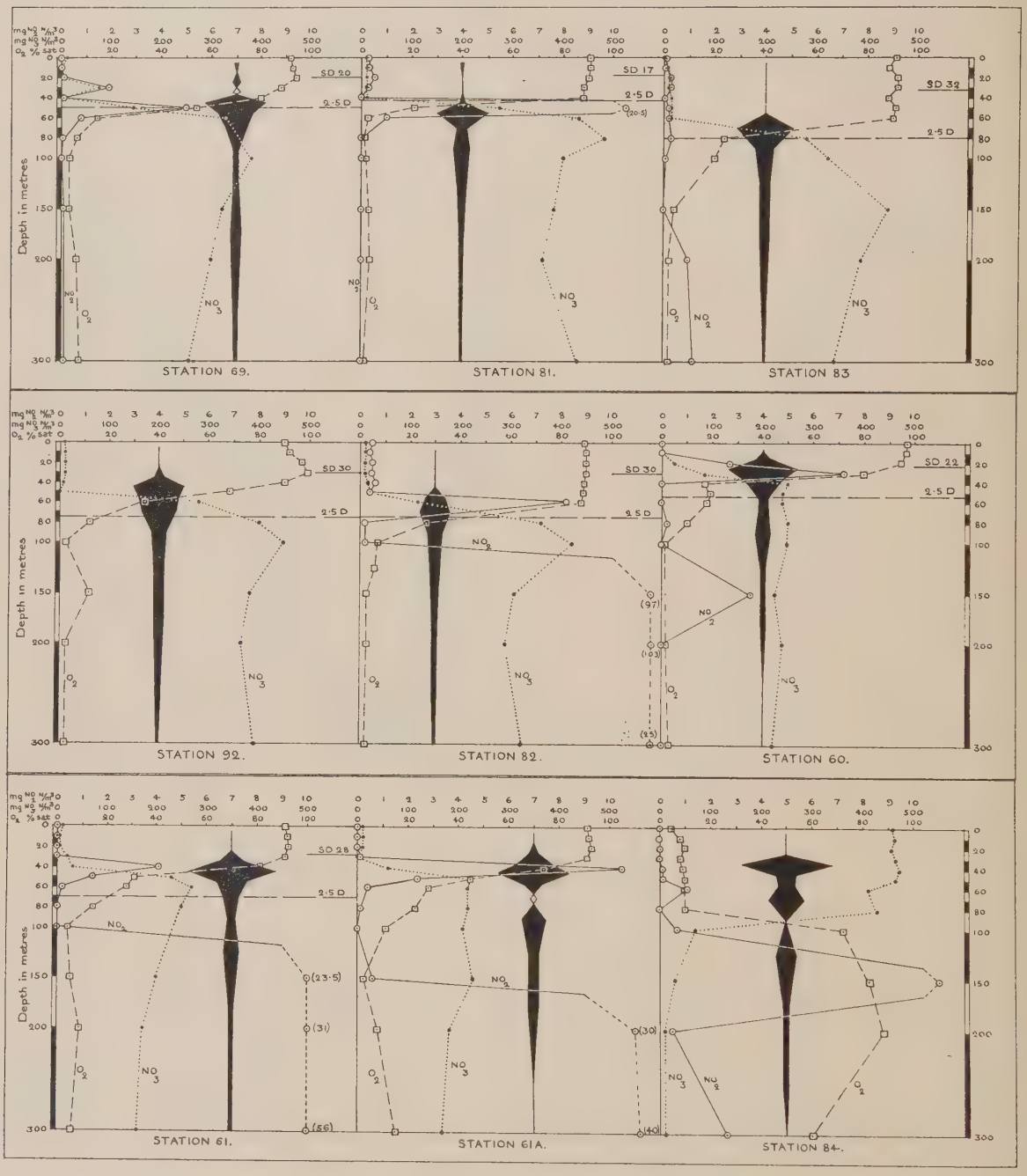
Muscat Section. Stations 65, 68, 76. November, 1933. (Text-figs. 6 and 7.)

Lying between 200 and 600 metres in the north-eastern half of the section (Text-fig. 7) is a body of water of relatively high salinity containing less than 300 mg. NO_3–N/m^3; this is water moving north towards the Persian Gulf from the broad extension of the continental shelf off Bombay. On the Arabian side at about 150 metres depth is a tongue of water of lower salinity and rich in nitrate coming out of the Persian Gulf. The deep water below 1000 metres has a much lower salinity and higher nitrate content (more than 500 mg. N/m^3), and is deep ocean water from the Arabian Sea.

Throughout the section there is a well-mixed surface layer from 30 to 40 metres thick, which is almost completely depleted of nutrient salts. The comparatively low oxygen figures indicate a sparse plankton owing to lack of nutrient salts. Nitrite shows some evidence of dying plankton in the discontinuity zone, and rather high values throughout the surface layer, probably owing to mixing by the wind.

Gulf of Oman Section. Stations 69–92. November to December, 1933.
(Text-figs. 8 and 9.)

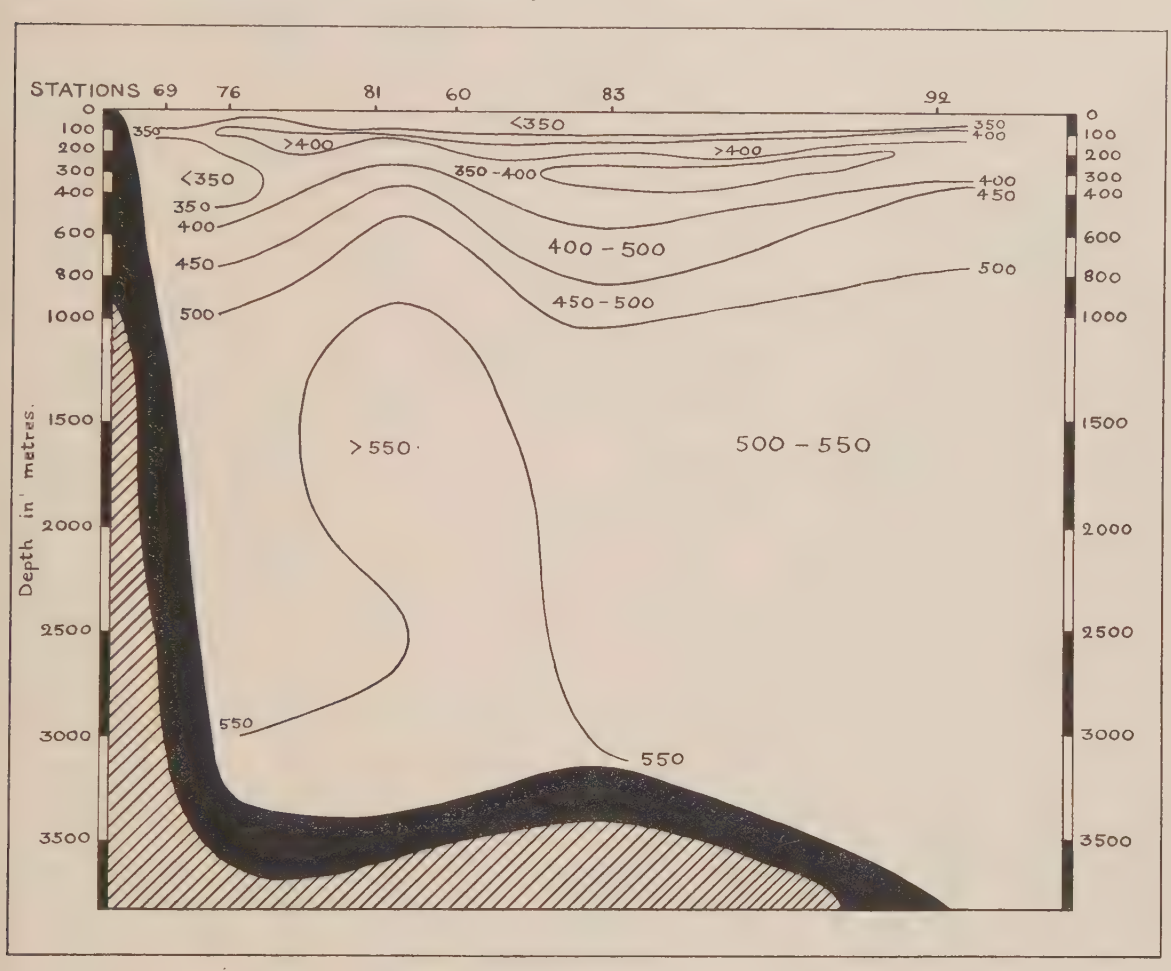
The longitudinal section through the Gulf shows the Persian Gulf water, containing more than 400 mg. NO_3–N/m^3, moving south at depths between 100 and 200 metres, and



TEXT-FIG. 8.

hugging the Arabian shore. Below it the nitrate-poor water from the Bombay region appears at some stations, but since it tends to keep to the Persian side, it does not appear at all of them (see Text-fig. 9).

The area appears to be one of comparatively scanty plankton production. Although more or less landlocked it receives no considerable supplies of nutrient salts from land, owing to the extremely small rainfall. The currents are of moderate velocity, but apparently produce little turbulence, so that although the stability is not very high, it is sufficient to prevent any considerable enrichment of the productive zone from below. The transparency was also low, probably owing to detritus carried out from the shallow waters of the Persian Gulf. At most stations nitrate was depleted almost down to the limit

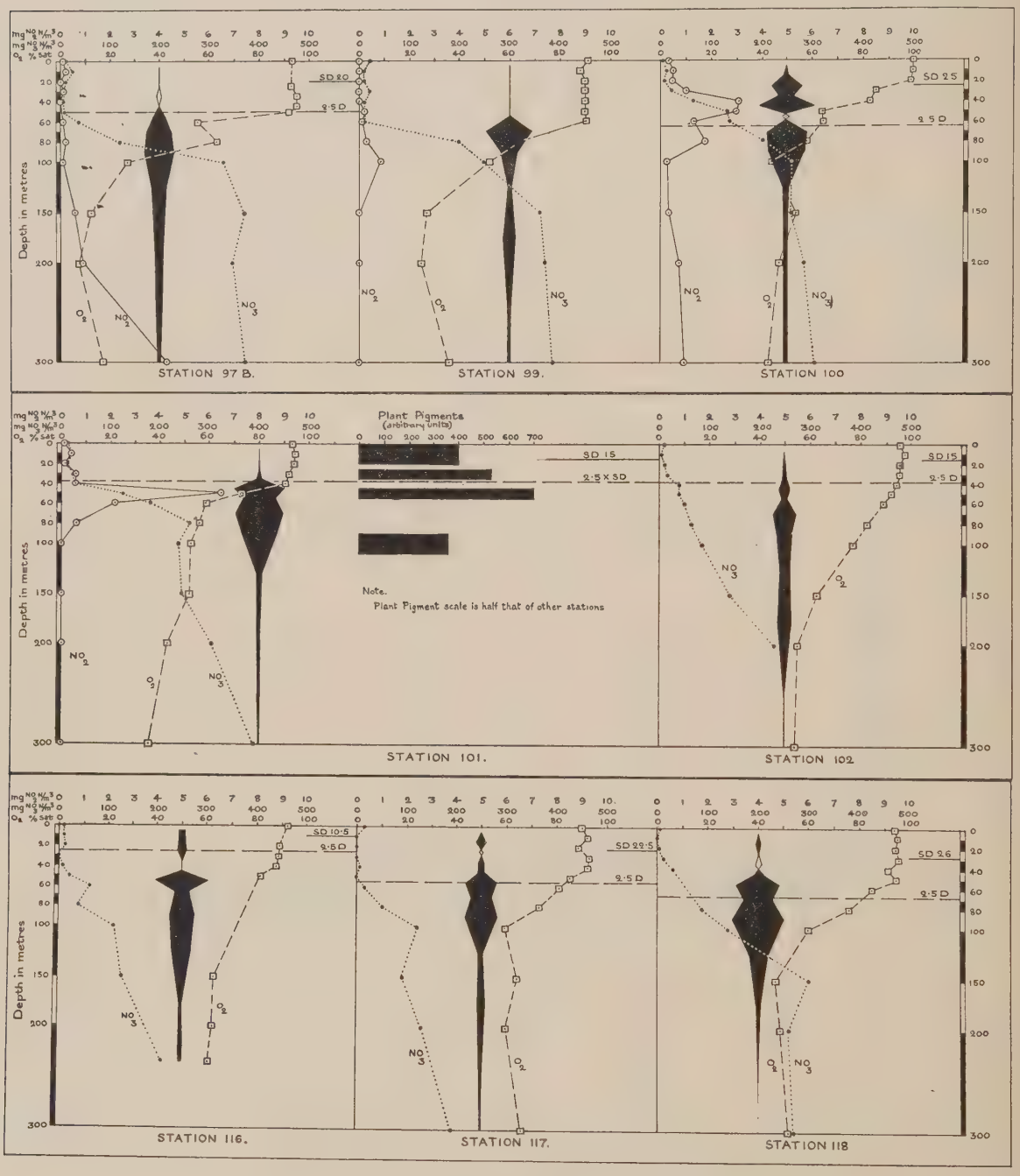


Text-fig. 9.

set by illumination, so that its lack was certainly limiting production. Neither oxygen nor nitrite figures (except Station 81) indicate any recent production on a large scale; if there is an outburst after the break-up of the SW monsoon, as we have seen in neighbouring areas, it must have been already long past by the time the expedition visited this area.

The phenomenally high nitrite concentrations recorded between 150 and 600 metres at Stations 61, 82, 84 and 92, and at 1000 metres at Station 83 (see tables in appendix to Vol. II), call for some comment, since some of these figures are far higher than anything previously recorded. Atkins (1930) reported finding 38·9 mg. NO_2–N/m^3 below the

thermocline in August off Plymouth, but the concentration at 200 metres at Station 82 (103 mg. N/m^3) was nearly three times this value. While no explanation can at present



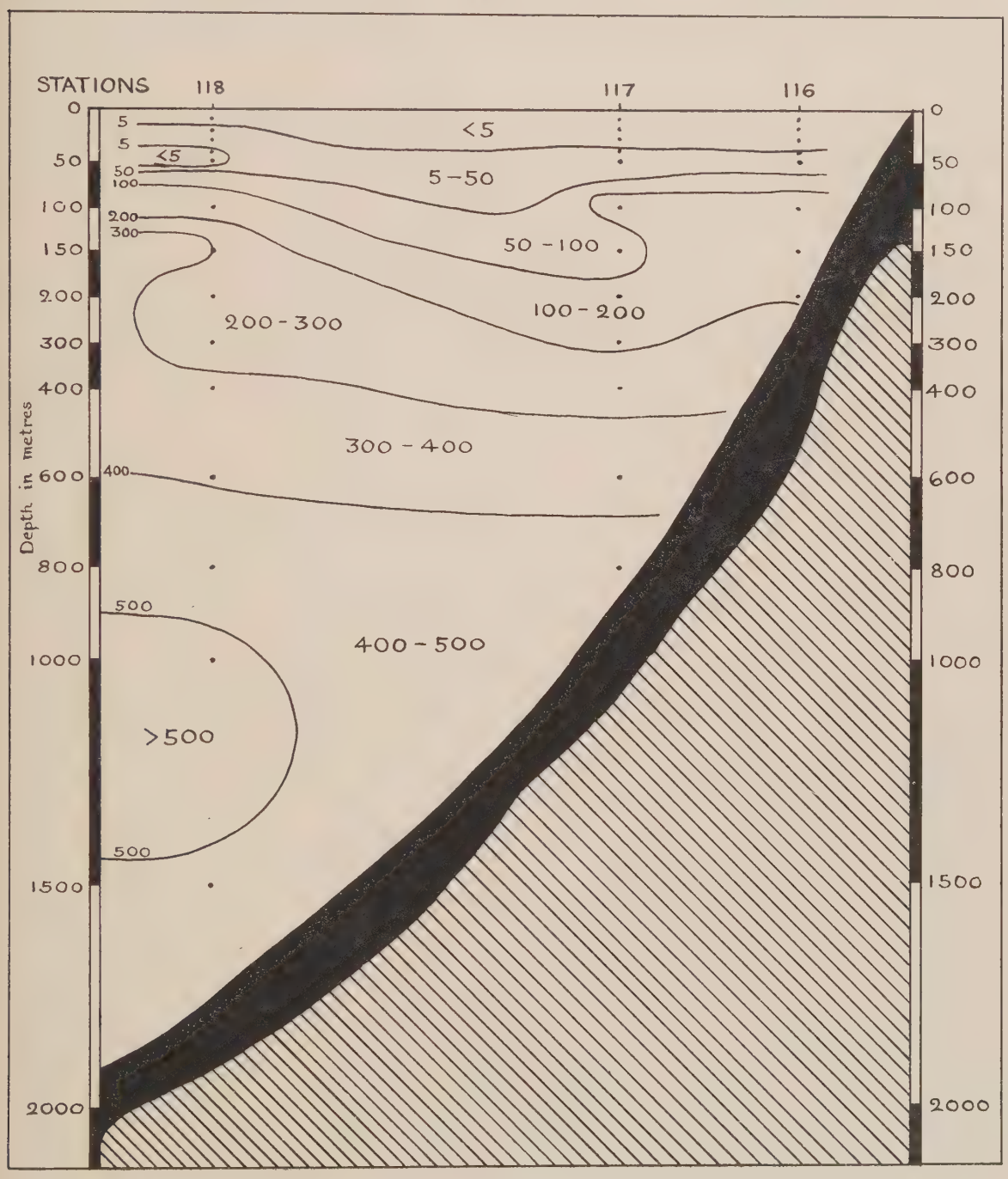
TEXT-FIG. 10.

be given, the fact that these stations lie in the neighbourhood of the "dead areas" described elsewhere in these reports by Col. Sewell (1935) is suggestive, but the connection cannot be regarded as established.

(4) *The Arabian Sea.*

Northern Section. Stations 92–118. December to January, 1933–4.
(Text-figs. 10 and 11.)

From Bombay as far as Station 100 conditions were fairly uniform. The surface layer was well mixed as far down as 50 or 60 metres, and almost completely depleted of nutrient salts. There does not seem to have been recent plankton production on any scale, and the area is probably always a poor one owing to lack of available nutrients.



Text-fig. 11.

At Station 100 we come into the sphere of the extremely rapid African coastal current, and enter the area of the richest plankton encountered by the expedition. During the SW monsoon season the northerly current along the African coast tends to move in a more easterly direction under the influence of the wind, and upwelling of deeper water results along the coast (see Matthews, 1926). The relics of this movement can be seen in the profiles at Stations 100 and 101 (see Text-fig. 10), although at the time when the Expedition visited the area the NE Monsoon was well established, and its influence is clearly seen in the stations closer to the shore. There the downward movement of water piled up against the coast is well shown by the small nitrate and oxygen gradients at Stations 102 and 116. The same tendency appears in a rather smaller degree at Stations 117 and 118 (see Text-figs. 10 and 11).

Unfortunately nitrite analyses could not be carried out for several of these stations, but where figures are available they resemble those of the Gardafui section in October, which, as we have already seen, was also a highly productive area. Again we find high values associated with a rapid change of density.

Southern Section. Stations 127–145. February to March, 1934. (Text-fig. 12.)

As in the northern section, the surface 40 or 50 metres had a comparatively low stability. Mixing by wind was more marked at the western side of the basin, where the NE monsoon is stronger, and has a greater fetch. However, as the compensation level lay at about 60 to 80 metres, well below the limit of such mixing, it caused no renewal of the supplies of nutrient salts.

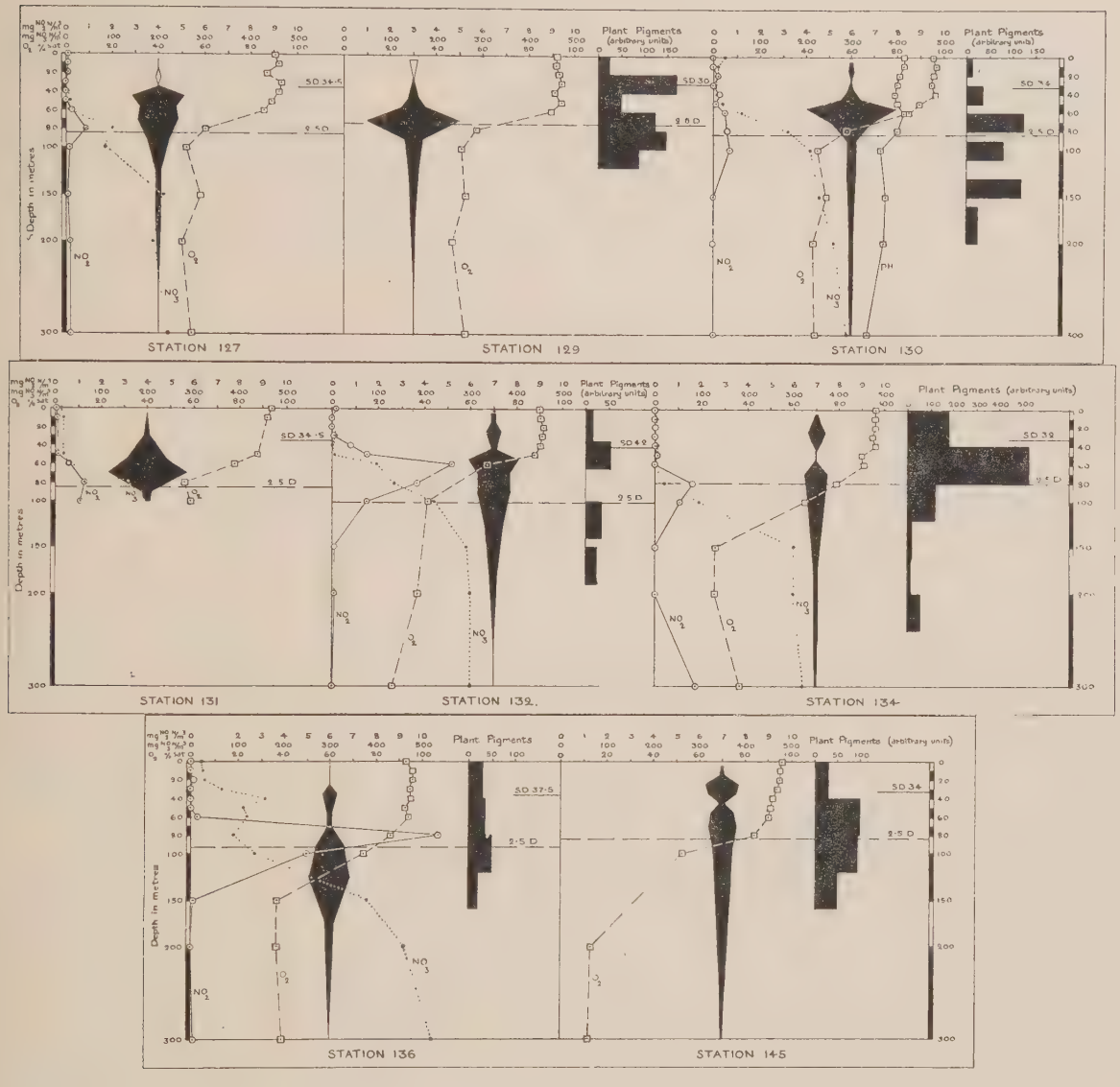
The open ocean stations appear to show stages following the end of a small phytoplankton outburst, the plankton having mostly sunk below the compensation point, and reached various stages of death and decay. On the whole the process is more advanced as we go east. Since this is the direction in which the expedition traversed the section, this may be due simply to the fact that the eastern stations were worked later than the western ones.

This outburst is probably conditioned by the return of calm weather, and the consequent greater stability in the surface layers, at the end of the SW monsoon season. With the advent of the NE monsoon mixing will increase again, but by this time the plankton is limited by exhaustion of its supplies of nutrients, and will be little affected. This was the condition at the time of observation.

Stations 129 and 134* both show higher plankton figures, especially near the surface (see Text-fig. 12). In the case of Station 129 this is due to upwelling under the lee of the Seychelles bank, and the upward movement is shown by the lower temperatures as compared with corresponding depths at Station 127. Station 134 might possibly be influenced similarly by the Maldive ridge, but the station is some way from the ridge, and the temperatures show no evidence of upwelling. On the other hand the salinity at the surface was distinctly lower than at Station 132. This indicates an influx of water from elsewhere, and Matthews (1926) has shown that this low salinity water is brought by the NE monsoon from the Bay of Bengal, Andaman Sea and the Malay region. Since enormous quantities of nitrate are brought down into the Bay of Bengal by the Ganges, it may well be that the water of this area is characterized by a relatively high nitrate content,

* Colonel Sewell informs me that this station was also very rich in small Copepods.

but unfortunately there are no analyses available to establish the point. The oxygen figures for this station (134) show clearly by the absence of any corresponding peak at that level that the phytoplankton found abundantly in the 40–80 metre layer did not originate there, but was sinking from above. Enrichment of the surface layers only will



TEXT-FIG. 12.

therefore explain the distribution found, and we are observing the same " aftermath " position that we have already discussed in the other stations.

If the magnitude of the nitrite peak is a true indication of the amount of planktonic decay which is going on or has recently occurred, Station 136, which was worked nearly four weeks later, shows a still more advanced stage of this process. The incomplete utilization of nitrate here is rather surprising. The position of the station on the ridge connecting the Maldives and the Chagos Archipelago suggests that there might be upwelling here too, but the relatively high stability between 100 and 150 metres makes this appear

unlikely—a conclusion which is borne out by the fact that the temperatures were slightly higher than those at corresponding depths at Stations 132 and 134. Possibly there had been regeneration of nitrate in the surface layers, or there may have been renewal by horizontal movements from the Bay of Bengal area, though the latter is not supported by the salinity. In any case unless some other factor, such as lack of phosphate, was limiting the plankton (in which case we need not postulate any influx of nitrate), any such renewal must have taken place very shortly before the time of investigation, since there was as yet no sign of a fresh plankton outburst.

Station 145 in the shallow water (494 m.) in Kardiva Channel shows the same type of conditioning as the other stations in the area. Unfortunately no analyses of the nitrogen compounds were made, but the absence of a peak in the oxygen curve indicates that the relatively large amount of phytoplankton had passed its period of maximum productivity.

Central Section. Stations 166–173. April, 1934. (Text-fig. 13.)

Excepting No. 173, the stations in this section show a large degree of similarity, the stability tending to decrease as we go westwards. The oxygen curves all show maxima well below the surface, and corresponding with plankton maxima where the figures are available. This indicates an active production of plankton in the layers between 20 and 60 metres, where the layer of optimum illumination probably lay, the light being almost certainly too intense near the surface. The transparency was high, and there was a correspondingly thick layer rich in oxygen.

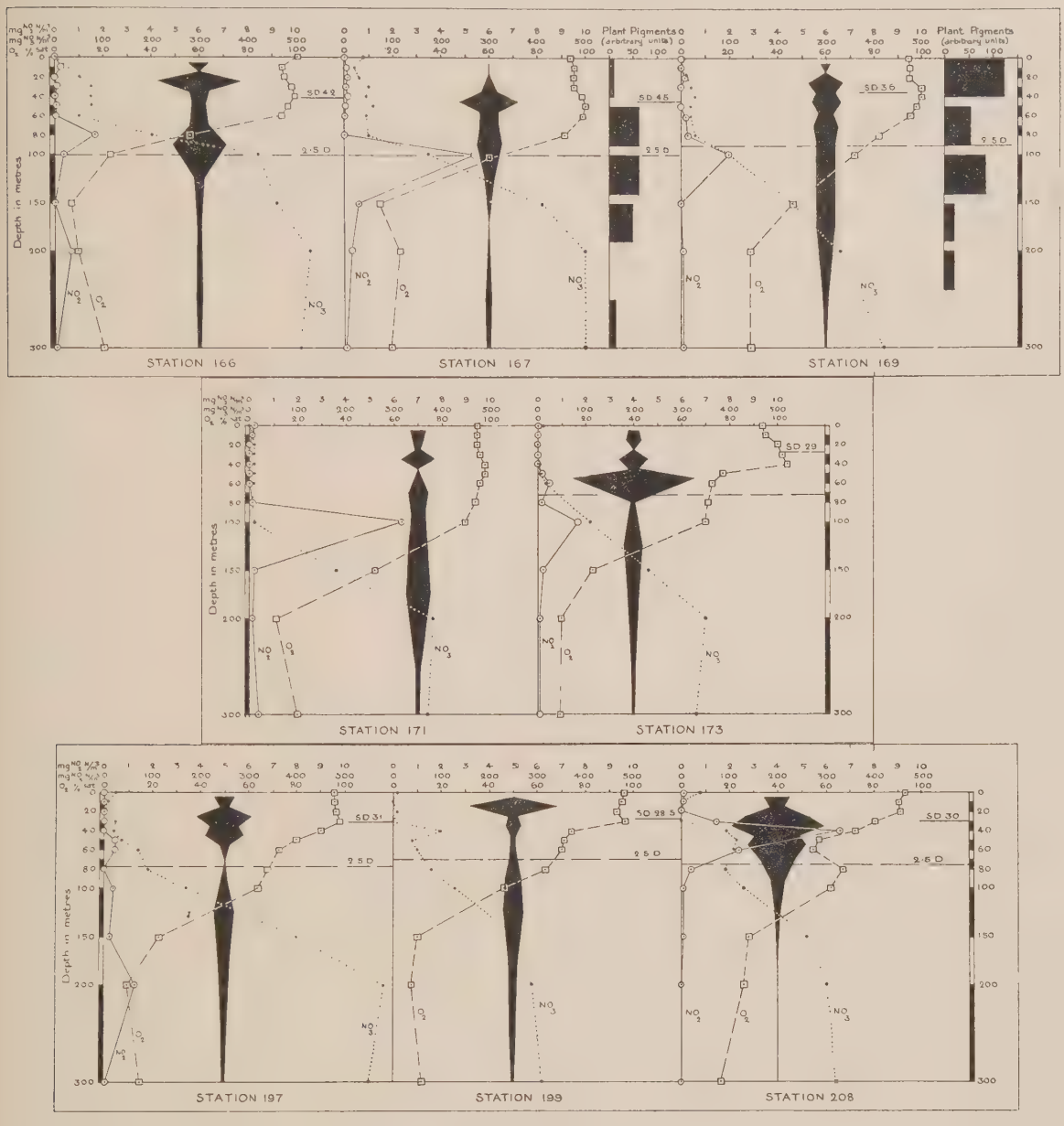
Station 166 is complicated by a layer of lighter water, which again probably originated in the region east of Ceylon, and was carried north by the coastal current of the NE monsoon season (Hornell and Ramaswami Nayudu, 1923). Utilization is incomplete even in the 20–60 metre layer, indicating probably an influx of water from a lower level elsewhere. Traces of the same influence are also to be found at Station 167, where, however, depletion had proceeded further in the uppermost 80 metres. The high nitrite at 100 metres is evidently connected with the relatively dense plankton population.

Station 169 is on the Carlsberg ridge, and its rich plankton is due to an upward movement of deep water. This movement is apparently a slow one, so that the supply of nutrients is not in excess of the consumption by the plankton. Thus its effect does not appear in the chemical picture, unless the slight nitrate peak at 40 metres is due to this cause.

At Station 171 a downward movement of water is indicated by the temperature, which was higher than at corresponding depths at Stations 167 and 169. Unfortunately the station was worked at night, and no Secchi disc reading could be taken, but it is unlikely that the water was clear enough to account for the almost complete depletion of nitrate as far down as 100 metres, which is probably also due to the sinking water. This same movement probably explains the fact that there was no great plankton production in progress, but a considerable amount of debris decomposing about the 100-metre level, since organisms would tend to be carried rapidly down below the compensation point.

Station 173 shows the profound influence of the strong currents in that region. The surface layer above the sharp boundary at 50 metres is water of relatively low salinity, which had come north with the African coastal current. The low oxygen and pH coupled with low nitrate at 50 metres probably indicate a rich zooplankton or bacterial population.

The Arabian Sea as a whole is dominated by the alternation of the two monsoons, and is therefore far more seasonal than most tropical oceans. From the end of May to mid-September the high winds and stormy seas of the SW monsoon prevail. We have unfortunately no observations during this period, and it is impossible to say to what

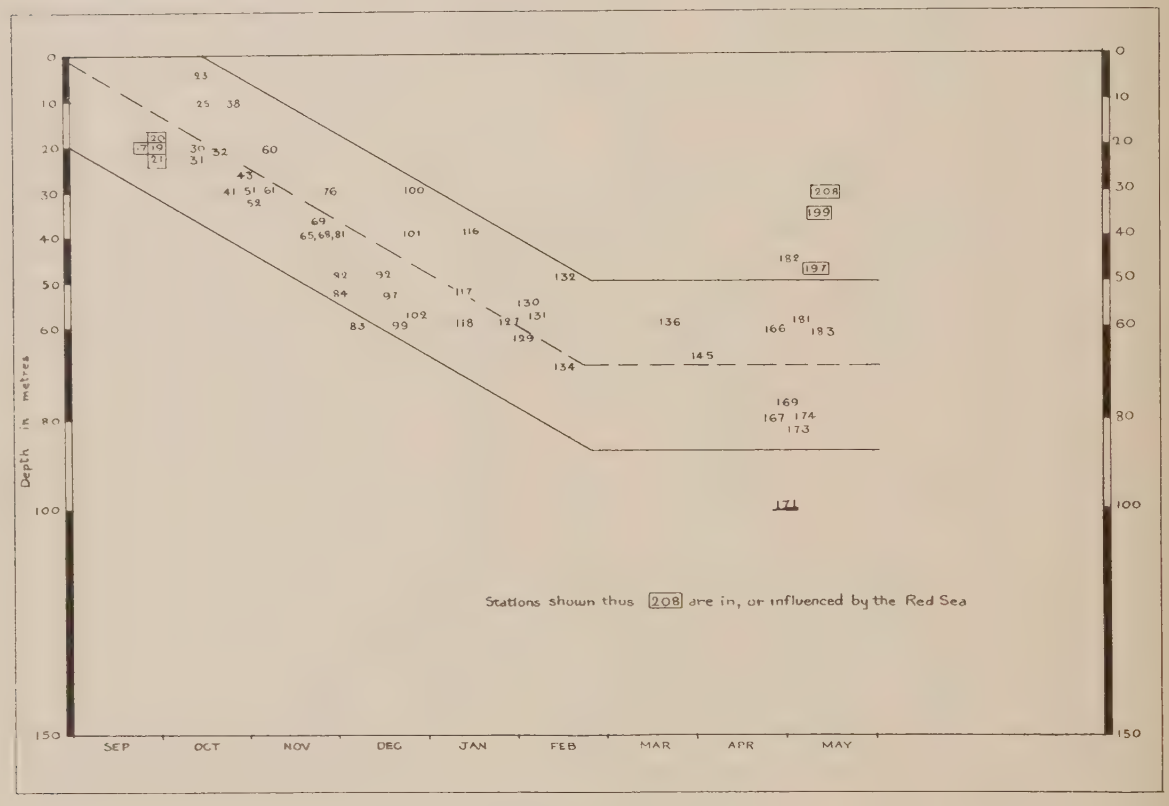


TEXT-FIG. 13.

extent stormy conditions retard the growth of plankton, or how deep mixing by the wind extends. It is, however, not improbable that a certain amount of nitrate and phosphate from the upper part of the thermocline is distributed through the surface layers. It should be noted here that the big outbursts of diatoms recorded in May off the Madras coast by Menon (1931), and off Calicut by Hornell and Ramaswami Nayudu (1923), are the direct result of the rains brought by the SW monsoon. They are perhaps partly due to dissolved

substances washed out from land, but are probably also the result of the lowered salinity and consequently increased stability of the surface layers. These outbursts are probably confined to the inshore waters, and would not be expected to occur in the open sea or in the Gulf of Aden region, where the monsoon brings little or no rain.

We may suppose that with the resumption of calm conditions in September, considerable growth of phytoplankton is initiated, but owing to shortage of supplies of nutrient salts this is mostly over by the end of January. From then on plankton is comparatively sparse except where upward water movements cause renewal of nutrient salts. This occurs principally in the strong currents near the African coast about 1–3° N.,



Text-fig. 14.

near the Seychelles bank, and in a smaller degree over the Carlsberg ridge, which runs from NNW to SSE, down the centre of the basin.

It must be emphasized that the picture given here of the plankton cycle must of necessity be incomplete, founded as it is largely on chemical data unsupported by direct seasonal observations.

Text-fig. 14 shows the influence of the calm period of the year in widening the limits of the productive zone. The depth of its lower limit, as estimated from the nitrate and oxygen figures, is here plotted against time, the points being indicated by the station numbers. The diagram shows that there is a general downward movement lasting till about the end of February, after which conditions remain more or less constant. Of the stations which fall outside the lines ruled on the diagram, the one in the Red Sea and the two at the western end of the Gulf of Aden (197. 199 and 208) clearly belong to a

different system, and are not comparable; at Station 182 we have already seen that there is a strong upward movement of water, and conversely a downward one at Station 171.

This deepening of the productive zone gives us the means of making a rough estimate of the phytoplankton crop. Thus in the five months between the end of September and the end of February there was, on the average, a fall of 55 metres in the lower limit of the productive zone. Now the average nitrate content of the 60-metre layer at eight stations at the beginning and eight at the end of this period was 290 and 30 mg. N/m^3 respectively. The change therefore represents the consumption of at least 200 mg. NO_3–N/m^3 in a layer of water 55 metres thick. Assuming nitrogen to form 0·5% of the wet weight of phytoplankton (Cooper, 1933*b*), this implies the production of 2·2 kg. of algal material (wet weight) per square metre of sea surface in 150 days, or a production rate of 14·4 gm./m^2/day.

This value is only to be regarded as the roughest of approximations. For the rich area at the mouth of the Gulf of Aden it is certainly too low, since no account of the upward water movements has been taken. On the other hand it is almost equally certainly too high for most of the Arabian Sea, since nitrate concentrations as high as 200 mg. N/m^3 are probably never found there within 20 metres of the surface.

When compared with other areas (see pp. 30, 31), the production rate is rather surprisingly high. Except for the upwelling areas, the figure reached probably represents the major part of the year's production, since there seems to be little further growth after the end of February owing to exhaustion of the supplies of nutrient salts, and growth is probably not very rapid during the stormy season of the year. Even if it represents a fair average for the whole annual production, it seems high compared with the English Channel figures (1·4 kg./m^2 as calculated by Atkins and Cooper), in view of the fact that the highest plankton population recorded (Station 101—an upwelling area) is only a little more than a tenth of the maximum recorded by Harvey (1934*b*) during the spring outburst, and the lowest (Station 132—average 31·5 units/m^3) is 25% lower than all but one of his records, and less than a tenth of the population generally present during a great deal of the year in the waters off Plymouth.* It is of interest that the figure for the rate of production is of the same order as that calculated by Seiwell (1935*a*) for the western part of the tropical North Atlantic. Whatever the value of the comparison, it is probably safe to say that the productivity of the tropical upwelling areas exceeds that of any temperate sea, while the open tropical ocean is by no means as barren as has commonly been supposed, and is indeed comparable in this respect with a region such as the English Channel.

V. THE NITROGEN CYCLE.

(1) Introductory.

It has long been known (Brandt, 1899, 1902, 1920; Harvey, 1920, 1928*b*, etc.) that the deep water of the great oceans contains a vast store of nitrate. As the most highly oxidized form of combined nitrogen this is also the most stable, and therefore the only form in which it persists for long periods in the sea. Since by far the greater part of the combined nitrogen in the sea exists in this form, it is perhaps not surprising that the

* It is probable that a larger proportion of the tropical plankton escapes the silk net than is the case in temperate regions.

photosynthetic organisms are adapted for its consumption as their principal source of supply, although some at least can also obtain their nitrogen from nitrite, ammonium salts, and even from simple organic compounds such as glycine (Schreiber, 1928 ; Braarud and Klem, 1931 ; Harvey, 1933).

As has been emphasized in the literature, the cycle nitrate→plants→nitrate is a closed one in the sea in general, except in a few places such as the Norwegian fjords and other land-locked seas, where the proximity of land provides an external source of supply. There is also in most places the possibility of supply by precipitation from the atmosphere, but this can be shown to be negligible. Thus on the basis of Braadlie's (1930) analyses of rain and snow, Braarud and Klem (1931) calculate that off the Norwegian coast the total annual addition of combined nitrogen from this source to the uppermost 10 metres of the sea would not increase the concentration in the water by more than 7 mg./m^3. It is possible that in some tropical regions with a high rainfall from thunderstorms this might be exceeded, but it is most unlikely that even as much as this small amount is added to the waters of most of the open oceans from this source.

So far we have two points in the cycle—nitrate and phytoplankton. We have seen from the fact that nitrate in temperate regions shows seasonal changes complementary to those of the phytoplankton population, and from the fact that plankton is especially abundant in regions where upwelling of deep water produces rich supplies of nitrate, that the link between these two stages is a close one. We must now consider the other side of the circle, and the stages which intervene between the death of the plankton and the return of its nitrogen to the stores of nitrate.

Here the main agents in the chain of transformations are the bacteria. A number of investigators have attacked the problem at various times, and with varying degrees of success, so that our knowledge is somewhat scattered, and the value of some of the earlier work is hard to estimate. Recently Waksman and his collaborators at Woods Hole have brought the methods of soil bacteriology to bear on the problem, and have contributed much to the understanding of previous work, which was often apparently contradictory. In a recent paper (Waksman, Hotchkiss and Carey, 1933) they have reviewed previous work, and summarized present knowledge of the part played by bacteria in the nitrogen cycle, and much of what follows here on the bacterial aspect of the problem is based on their paper.

On the death of the plants, or of the animals which feed on them, the complex organic compounds into which they have built up the nitrogen assimilated as nitrate are attacked by bacteria, which break them down into simpler organic compounds such as amino-acids and amines, and finally to ammonium compounds. This ammonia is then usually oxidized by other bacteria first to nitrite and finally to nitrate. So much for the direct cycle, but there are also various side branches. Thus the nitrate, if not already consumed by phytoplankton, may, under certain conditions, be reduced by other bacteria to nitrite, ammonia or gaseous nitrogen or its oxides. Other bacteria in the presence of carbohydrates may compete with the plants for the available inorganic nitrogen, and convert it into organic complexes. Finally, yet other bacteria fix atmospheric nitrogen, and build it up into the organic compounds of their own body substance.

Bacteria are thus concerned with five different processes all involving the transformation of nitrogen compounds : (1) Liberation of organically bound nitrogen as ammonia ; (2) oxidation of ammonia to nitrite and nitrate ; (3) reduction of nitrate to nitrite and

free nitrogen; (4) synthesis of inorganic nitrogen into organic compounds; (5) fixation of atmospheric nitrogen.

(2) Ammonification of Organic Matter.

This aspect of marine bacteriology has been very much neglected, the interest among earlier investigators having been mainly focused on the nitrifying and denitrifying forms, or on the distribution of bacteria of all sorts. The most valuable contribution is that of Waksman, Carey and Reuszer (1933). They found that zooplankton material was rapidly decomposed, with liberation of about half the nitrogen as ammonia, in both sea-water and mud media. More CO_2 was produced in the mud cultures, and it is suggested that, whereas the proteins are decomposed equally in both media, other constituents poor in nitrogen, such as chitin, or nitrogen-free, such as certain fats, are more readily attacked by the bacteria of the mud. The decomposition of algal material was found to depend similarly on its composition. Material rich in nitrogen, such as *Ulva* (*ca.* 2% N dry weight, salt-free basis), decomposed both in water and in mud. On the other hand *Fucus* (less than 1% N dry weight, salt-free) was only very slowly attacked in the water, but decomposed rapidly in the mud medium. Addition of inorganic nitrogen sources favoured the decomposition of nitrogen-poor material in the sea-water, both nitrate and ammonium salts being readily usable to supply the nitrogen required for cell synthesis by the bacteria.

Thus decomposition of algal residues poor in nitrogen may result in a minimum of available nitrogen free in the water, while animal residues produce abundant supplies of ammonia. The bacteria responsible are present both free in the water and in the sea bottom.

Our knowledge of the distribution of ammonia in the sea is somewhat scanty. Since the distillation methods used have been shown to be liable to form ammonia by hydrolysis of organic compounds, the results reported by earlier workers cannot be implicitly relied upon. However, such data as we have tends to support the view that ammonification is largely a surface and to some extent also a bottom phenomenon.

Thus Brandt (1920) showed that in the tropical Atlantic ammonia exists principally in the subsurface layers, and that it is much less abundant in regions where upwelling water brings tongues of water up from the depths. Böhnecke, Hentschel and Wattenberg (1930), in the water west of Iceland, found the ammonia profile to be an almost exact mirror image of the nitrate profile, indicating that consumption of nitrate in the surface layers by the plankton is followed by ammonification in the same layers. Robinson and Wirth (1934*a*) in the Puget Sound region found a rather irregular distribution, which differed in that the maximum tended to be well below the surface. Similar results are reported by Braarud and Klem (1931), who found that off the Norwegian coast there was a tendency for a maximum in the 40–70-metre layer and a minimum at the surface. In the deep water off Vancouver Island (Robinson and Wirth, 1934*b*), what ammonia there was, was nearly all in the uppermost 100 metres, the average values being 20 mg. NH_3–N/m^3 at the surface and 2 mg./m^3 at 1500 m. There the shallow inshore stations contain more ammonia, and an especially high concentration was found at the bottom of a submarine gully, owing to stirring up of the mud.

Cooper's (1933*a*) results for the English Channel also support the same view. In

winter he found a maximum near the surface, a minimum in mid-water, and intermediate values at the bottom. In spring the surface values decrease, which it is suggested is due partly to consumption by the plankton, and partly to lack of supplies of organic matter owing to the sparse plankton production during the winter months. Later a general rise occurs in summer and autumn towards a maximum of about 30 mg. N/m^3. High ammonia concentrations are often found near shore owing to land drainage, especially where the sewage of a big town is discharged.

Unfortunately, owing to lack of time and laboratory space on board, ammonia determinations were not found possible on the John Murray Expedition, so that little is known about the distribution of ammonia in the open oceans. Such attempts as were made indicated that where any ammonia was present at all, it was in the uppermost 100 metres.

In general, then, the distribution of free ammonia in the sea accords with what we might expect from the bacterial distribution reported by Waksman, Carey and Reuszer. Ammonification is carried on immediately on the death of the organisms by bacteria which are present among the plankton. In shallow water a certain amount of nitrogenous organic matter may reach the bottom undecomposed, and ammonification may proceed there, but in deep water the organic nitrogen is nearly all liberated before reaching the bottom.

(3) Nitrification.

The process of oxidation of ammonia through nitrite to nitrate has been the subject of a good deal of research, but since the bacteria concerned are very sensitive to environmental conditions, and are not easily cultured, the negative findings of some of the earlier investigators must be regarded with some suspicion.

The presence of nitrifying bacteria in the sea was first suggested by Vernon (1898), and was demonstrated in mud, but not in the water of the Baltic by Baur (1902). Gran (1903) failed to confirm this in the Norwegian fjords, finding them only close to the shore, and Nathansohn (1906) found none in the Gulf of Naples.

Brandt (1902) had previously argued that if nitrate comes into the sea from the atmosphere or from land, we should expect to find it most abundant in the surface layers which is contrary to the facts. He therefore argued from its abundance in deep water that nitrification occurs chiefly at or near the bottom.

Thomsen (1910) found nitrite-forming bacteria abundant in all bottom samples from Kiel Fjord, Heligoland, and the Gulf of Naples, but absent from sea-water, plankton or fixed algæ. Nitrate-formers were also found in mud samples, but only close to shore. The organisms responsible were isolated, and were found to be morphologically identical with the *Nitrosomonas* and *Nitrobacter* isolated by Winogradsky from soil, and to have similar high temperature optima. He attributed Nathansohn's negative results to unsuitability of the culture medium.

Issatschenko (1908, 1914) found nitrifying bacteria in the water of the Arctic Ocean ; they were also found in bottom material from the Catherine coast, from the Northern Ice Sea, and from the high seas, but were absent from surface water. He found only nitrite-formers in the bottom material. Later (1926) he reported that nitrifiers were more abundant in sandy and shell-rich bottoms than in clay.

Lipman (1922) concluded that, although absent from the water of the open sea,

nitrifying bacteria are normally present in the sea bottom. Similar findings were reported by Harvey (1925, 1926, 1928*b*).

On the basis of these results Brandt (1927) considered it established that bacteria capable of oxidizing ammonium salts are completely lacking in surface water, but are present in the sea bottom.

Waksman, Hotchkiss and Carey (1933) as a result of a critical series of experiments reached much the same conclusion, namely that free sea-water, especially surface water, contains either no nitrifying organisms at all, or very few. On the other hand the sea bottom, whether mud or sand, contains an active population. Nitrite-formation in media containing ammonium sulphate was easily demonstrated, but it was not found possible to obtain formation of nitrate by direct inoculation into media containing nitrite. However, after longer incubation of the nitrite-forming cultures, nitrate formation could be shown, and inoculation of nitrite containing media from such old cultures gave rapid nitrate formation. The fact that it was much easier to demonstrate the formation of nitrite is attributed to the specificity of the organisms and the conditions of cultivation. Further results supporting their conclusions are reported by Carey and Waksman (1934).

As already mentioned, the abundant nitrate of all deep ocean waters points strongly to the bottom as at any rate the principal site of the nitrification process. In shallower water where there is vigorous mixing, at least at some time of the year, it is possible that these bacteria get carried up into mid-waters. This is probably the explanation of Cooper's (1933*a*) conclusion that nitrification proceeds throughout the water column, and his observation that an increase of ammonia is usually followed after an interval by an increase in nitrite, and later by an increase in nitrate, supports this view.*

Deacon (1933, p. 234) remarks that nitrite is " a stage in the formation of nitrate from animal decomposition ", and that " its absence from deep waters with the greatest phosphate and nitrate is surprising ". It is, however, probable that nitrate formation in the sea is a very much more rapid process than it was in Waksman, Hotchkiss and Carey's cultures. The nitrate-forming organism isolated by Thomsen (1910) had a high temperature optimum, but there is no reason to suppose that it is the only organism present, or even a typical member of the population. It is indeed almost incredible, in view of the generally low temperatures prevailing at the bottom of the sea, that there are not other nitrifying bacteria in the marine muds, which are more closely adapted to their environment than this form whose characteristics fit it for life at the temperatures of land soils. The viability of such organisms would be likely to be low under laboratory conditions, and it is easy to see how culture experiments might fail to show rapid nitrification as a characteristic of marine muds. Furthermore, since nitrification is proceeding in the mud, the products presumably escape only by diffusion into the overlying water. Such a process is bound to be slow, and would allow time for the completion of the oxidation. Even if some nitrite does diffuse out into the water, its presence would usually escape notice, since the deepest samples collected are usually several metres above the bottom.† The general absence of nitrite in deep waters cannot therefore be held to indicate

* See also Atkins (1930), where the same view is put forward.

† Rakestraw (1933) records finding both nitrate and nitrite much more abundant in water extracted from mud brought up in a grab than in water collected 3–4 metres above; the ratio of the two was much the same in both cases.

any different nitrification process. This is confirmed by its presence in the bottom water of shallow, relatively unstable seas such as the English Channel, where any which does escape from the mud will be carried up into the layers which can easily be sampled.

(4) Reduction of Nitrate, and Denitrification.

Owing to the early recognized importance of nitrate as a raw material for production of plankton, considerable interest has centred on those bacteria which destroy it. In considering the reported work of the earlier investigators, it is important to remember that there are three groups of bacteria included under this general heading: (*a*) those which reduce nitrate to nitrite only, (*b*) those which reduce nitrate and nitrite to ammonia, and (*c*) those which reduce nitrate and nitrite to free nitrogen and gaseous oxides of nitrogen. Only the last group are properly called " denitrifiers ", since they alone cause loss of nitrogen from the system.

Nitrate-reducing bacteria were demonstrated in the sea by Beijerinck (1890), Fischer (1894), Russell (1893) near Woods Hole, and Vernon (1898) in the Gulf of Naples.

In 1899 Brandt put forward his famous theory that the nitrate-reducing bacteria control the supply of available nitrogen, and so the production of plankton. At that time he believed that the supply of nitrate came entirely from land, and that but for these bacteria it would soon reach a concentration in the sea which would be lethal for the plants and animals. It was observed that the plankton-rich temperate and arctic seas were also rich in nitrate, and he attributed the poverty of tropical oceans to their low nitrate content. This in turn he supposed to be due to the greater activity of denitrifying and nitrate-reducing bacteria at tropical temperatures.

The publication of this theory attracted interest to the problem, and many investigators reported the discovery of denitrifying bacteria in various parts of the world—*e. g.* Baur (1902), Nathansohn (1906), Kühl (1908), Gräf (1909), Issatschenko and Rostewzew (1910), Drew (1910), etc.

Feitel (1903) showed their presence in great abundance at all depths, but especially on the bottom or in water near it, where organic matter was abundant. He isolated forms in pure culture which were active denitrifiers, especially at low temperatures. The general occurrence of bacteria of these types, together with the fact that nitrate decreased with rising temperature towards the surface in tropical seas, was held to support Brandt's hypothesis.

Gran (1901) isolated several nitrate-reducing bacteria, but he found that only close to shore, where abundant organic matter and nitrate are present, do denitrifiers play any important part in the nitrate economy of the sea. He considered Brandt's theory untenable.

Issatschenko (1914) found denitrifying bacteria capable of growing at very low temperatures (1–3° C.) in the North Polar Sea. He concluded that forms reducing nitrate to nitrite and ammonia were widely distributed, normal inhabitants of the sea, but he considered it doubtful whether the true denitrifiers, which are also widely distributed in northern seas, cause any considerable loss of combined nitrogen. Since they are active at low temperatures, and warm seas are also rich in nitrates in the lower layers, he also denied the validity of Brandt's views.

Cranston and Lloyd (1930) investigated the metabolism of a denitrifying bacterium

isolated from the sea (see also Lloyd and Cranston, 1930, and Lloyd, 1931). Their results can be explained by the assumption that the reaction proceeds in three stages, with intermediate formation of hyponitrite, thus :

$$2KNO_3 - O_2 = 2KNO_2,$$
$$2KNO_2 - O_2 = K_2N_2O_2,$$
$$2K_2N_2O_2 - O_2 + 2H_2O = 2N_2 + 4KOH.$$

Since each stage is completed before the next is begun, four-fifths of the oxygen available in the nitrate can be obtained without liberation of any nitrogen. Thus in the presence of abundant nitrate no gas is formed. The oxygen obtained by the organism goes to form CO_2, which combines with the KOH produced at the last stage, so that the medium goes acid at first, and finally alkaline.

It is thus possible that many of the forms which have been found only to reduce nitrate as far as nitrite are really facultative denitrifiers if given suitable conditions. Since, however, these include absence of oxygen as well as presence of a suitably small quantity of nitrate, it is unlikely that they are at all commonly realized in the sea.

That the bacterium studied by Cranston and Lloyd is not the only one concerned is indicated by its high temperature optimum (35° C.), since Issatschenko showed active denitrification at very low temperatures. It is also shown by the fact that in many cases denitrification is said to be favoured by good aeration (*e. g.* Gran, 1901), and by Waksman, Hotchkiss and Carey's (1933) experiments, where active denitrification was observed under aerobic conditions.

The latter authors confirmed Issatschenko's conclusion that forms reducing nitrate to nitrite are abundant in the water, and even more so in the mud. On adding an energy source such as algal material denitrification could easily be demonstrated, especially in mud cultures, but they consider it unlikely that this occurs to any great extent in the sea.

Denitrification and the reduction of nitrate to nitrite or ammonia are therefore processes requiring very special conditions. Not only is an abundant supply of an easily oxidized energy source, such as carbohydrate, necessary, but also an excess of nitrate over the requirements of the plants and the saprophytic bacteria of decay. As pointed out by Gran (1912*a*), such conditions may occur in enclosed fjords, where the organic matter is high at the bottom. In general the surface layers, which are richest in suitable organic matter, are too poor in nitrate, while the bottom water, which is rich in nitrate, contains comparatively little organic matter ; what there is, like that of the mud, is mostly of an unsuitable type such as pentosans and polyuronides, which are only attacked by specific bacteria (Waksman and Carey, 1933 ; Waksman, Carey and Reuszer, 1933 ; Waksman, Carey and Allen, 1934).

There is, however, one level in the sea where a compromise may be found—at the lower limit of the photosynthetic zone. Here there is abundant organic matter,* and since the growth of phytoplankton is prevented by lack of light, there is also ample nitrate available. We may therefore anticipate that in areas where there is any considerable production of plankton, the presence of nitrate-reducing bacteria would

* This is supplied partly by the carbohydrate secreted into the water during the life of the plant cell (Braarud and Klem, 1931), and partly by the disintegration of dying organisms.

be reflected in a high nitrite concentration at this level. As we have already seen, this is normally the case in the open sea.*

(5) Bacterial Synthesis of Organic from Inorganic Nitrogen.

Of this type of bacterial activity comparatively little is known. It probably comprises rather an aspect of the metabolism of various forms than any one group of bacteria.

We have already seen (p. 61) that the bacteria which break down organic residues can, when the substrate they are attacking is poor in nitrogen, obtain supplies for the synthesis of their own body substance from the inorganic nitrogen compounds in the water. Bacteria of this type, which decompose specific algal constituents, have been studied by Gran (1901), and recently by Waksman, Carey and Allen (1934), but little attention has been paid to their nitrogen metabolism. Besides these specialized forms there are probably many generalized saprophytes, which live among the plankton on the carbohydrates and other products of metabolism, obtaining their nitrogen partly from inorganic sources.

(6) Nitrogen-fixing Bacteria.

Benecke and Keutner (1903) were the first to demonstrate both types of nitrogen-fixing bacteria in the sea. The aerobic *Azotobacter* they found mainly on plankton, and the anaerobic *Clostridium* mainly in deep water and in the sea bottom. Keutner (1905) concluded that they are normal inhabitants of the sea, having been found in the North Sea, Indian Ocean, on the African coast, and in the Malay Archipelago. This has been repeatedly confirmed by later investigators, *e. g.* Keding (1906), Benecke (1907), Issatschenko (1908, 1914), Bavendamm (1932), Waksman, Hotchkiss and Carey (1933). Their especial abundance in the slime on *Zostera* and the shore algæ as well as among the plankton has led to the suggestion of symbiosis, but there is at present no means of verifying this.

Like the denitrifiers, they require a source of energy as well as supplies of other nutrients such as iron and phosphate. Of the quantitative aspect of their part in the nitrogen cycle nothing is known.

(7) Inorganic Influences.

Besides the activities of the various types of bacteria known to be present in the sea, it has been suggested that certain transformations may be brought about by inorganic means.

Thus Darnell-Smith (1894) suggested that the oxidation of ammonia to nitrite and nitrate might be carried out by oxygen given off in a " nascent " state by photosynthetic organisms. He described experiments in which sea-water, allowed to stand, increased in ammonia content " by decomposition of organic matter ". If *Ulva* was then put into the water, in the light a rapid fall in ammonia occurred, in the dark a less rapid rise. A very much slower fall in ammonia content was caused by aeration of the water without any *Ulva*. That the rise in ammonia was due to the decomposition of organic matter is probably correct, and supports the view, already expressed, that ammonifying bacteria

* Since writing the above, I have discovered that Rakestraw (1933) suggests the same origin for the nitrite found about the 40-metre level in the Gulf of Maine.

are generally present. But the inference that the fall in the presence of *Ulva* in the light was due to oxidation by nascent oxygen seems unwarranted, since it is well known that ammonia can serve as a nitrogen source for algæ, and as the water was probably surface water, it may well have contained little nitrate. The fall in ammonia on aeration is also easily explained. If the air used was freed of ammonia, as was presumably done, the partial pressure of ammonia in the alkaline sea-water would be greater than in the air, with resulting loss to the air-bubbles as they passed through the water. Finally there is no evidence that the oxygen given off by plants is in a " nascent " condition.

Zobell (1933) has suggested that the same reaction may proceed photochemically. He found that flasks of sea-water to which ammonium sulphate had been added, if kept in the sun for a fortnight, showed a rise in nitrite and nitrate and a corresponding fall in ammonia, while controls kept in the dark remained unchanged. The reaction was unaffected by Berkfeld filtration, accelerated by manganese dioxide, imperceptible in distilled water or artificial sea-water, and abolished by autoclaving at 120° C. for half-an-hour. The effect was attributed to the ultra-violet rays, a mercury arc being more effective than sunlight. The water used is said to have given no growth of nitrifying bacteria on inoculation into Winogradsky's solution. The experiments so far reported are of a preliminary character, but light does appear to have influenced the reaction. The absence of nitrifying bacteria cannot be held to have been established, though the fact that Berkfeld filtration did not affect the result indicates that they were not the principal agents. This and the effect of autoclaving suggest the possibility of some thermolabile catalyst of an enzymatic character. A similar suggestion has been made by Kreps (1934).

Another photochemical reaction, again tending to form nitrite, but in this case by reduction of nitrate, has been described by Thiele (1907), and by Moore and Webster (1919).

In any case such reactions depending mainly on the ultra-violet can be of no importance except in the extreme surface layers, since it has been shown (Atkins, 1932) that the ultra-violet is very rapidly extinguished in the sea owing to scattering by small particles. Since the ammonia and nitrate concentrations in these layers are usually small, the magnitude of the effects cannot be large enough to play any considerable part in the nitrogen cycle. This is borne out by the fact that it is quite exceptional to find any demonstrable amount of nitrite at the surface.

(8) Conclusions.

Having briefly reviewed the various agencies causing transformation of nitrogen from one form to another, we are in a position to draw some conclusions on the cycle as a whole.

Nitrate forms the main store of combined nitrogen in the sea. In the algal cells it is built up into organic compounds—principally the proteins of their protoplasm. If the algæ are then eaten by animals the plant proteins are broken down by the digestive juices, and the amino-acids resynthesized into the proteins of the animal body. The animal phase may be prolonged through several stages, as small animals are eaten by larger ones, and they by larger ones again. Throughout there is a continual return of a proportion of the nitrogen to the sea as urea or ammonium salts in their excreta.

On the death either of the uneaten plant cell, or of any member of the animal chain, we start a series of bacterial transformations. The complex compounds are first of all broken down by the bacteria of decay through simple amino-compounds to inorganic ammonium salts. The ammonia, whether formed by these ammonifying bacteria, or set free in animal excreta, is then attacked by nitrifying bacteria, and oxidized, first to nitrite, and finally to nitrate.

In its simplest form the cycle is now complete. The synthesis of the nitrate nitrogen into the proteins of living matter proceeds in the illuminated zone, and the same is true in the main of the conversion from plant to animal proteins. The ammonification process appears to be carried out mainly in the same region by bacteria which live among the plankton, and partly also by bacteria living in the sea floor, and attacking the corpses which sink there from above. Nitrification, on the other hand, occurs almost entirely in the mud at the bottom. How the ammonia formed near the surface comes under the influence of nitrifying bacteria is not altogether clear. In the cooler parts of the world it may be carried down by convection currents, as suggested by Böhnecke, Hentschel and Wattenberg (1930), but this cannot occur over vast areas of the tropical and sub-tropical oceans. It may be that nitrification also proceeds in the sub-surface layers to a much greater extent than the experiments would suggest ; the rapid rise in nitrate concentration at the lower limit of the productive zone lends some support to this view. Alternatively it is possible that ammonification goes on in deep water to a greater extent than has been supposed, and is masked by the rapid nitrification which follows. This would explain the presence of abundant nitrate in tropical deeps, but not the absence of large quantities of ammonia in the sub-surface layers. It is known that ammonia can serve as a source of nitrogen supply for the phytoplankton, but that it does not do so to any great extent in fact is indicated by the absence of pronounced seasonal fluctuations in the ammonia of the sea, such as are well known for nitrate, phosphate and silicate. It is, however, possible that such consumption of ammonia does occur in the tropics, where the plankton has a rather different composition, and where the absence of any marked seasons would make such consumption less easy to demonstrate. As we have at present no knowledge of the nitrogen metabolism of tropical plankton organisms, especially the Coccolithophoridæ, or of the rate at which the bacterial transformations proceed at different depths and at different seasons, it is impossible at present to reach a satisfactory answer.

The activities of the other types of bacteria are probably of relatively minor importance, so far as the nitrogen cycle is concerned, although, as Waksman and his colleagues have shown, some of them are the main agents in the formation of " marine humus ". How far the competition of the saprophytic forms living in the surface layers affects the production of phytoplankton it is impossible to say at present, but there is no doubt that they form a large part of the food of the smaller zooplankton (*cf.* Clarke and Gellis's [1935] experiments on the nutrition of Copepods).

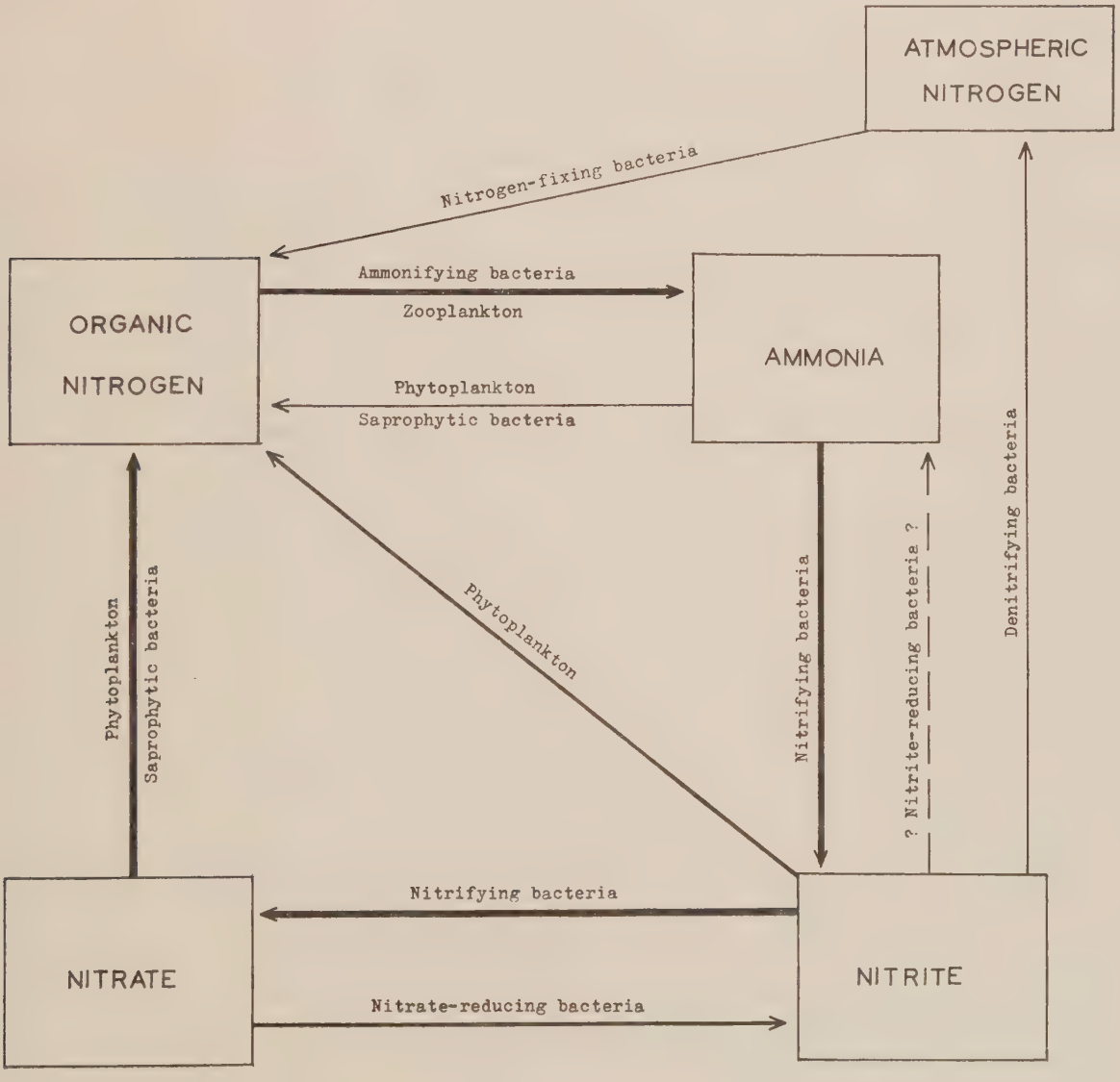
Since nitrite is readily assimilable by the plants, the nitrate-reducing bacteria, which we have seen to be active at the lower limit of the productive zone, do not affect the supply of available nitrogen.

Further evidence that the activities of the denitrifying and nitrogen-fixing types are not quantitatively of any great importance is given by Redfield (1934). Comparing the concentrations of nitrate and phosphate in sea-water from various places and depths with

simultaneous observations of the oxygen and carbon dioxide (calculated from the pH), he shows that carbon, nitrogen and phosphorus vary together in the sea in the approximately constant ratio C : N : P : : 140 : 20 : 1 atoms or 100 : 16·7 : 1·85 gm. The correspondence for open sea data from polar, temperate and tropical regions is good, but the ratios are variable in regions subject to land influences and disturbance by water movements, such as the English Channel and the Norwegian Fjords. The average of a number of plankton analyses taken from various authors gives the ratios C : N : P : : 100 : 15·4 : 1·88 gm. for the composition of plankton.

The general agreement indicates that the amounts of nitrate and phosphate present in the sea are controlled, in the main, by the same agency—the consumption by the phytoplankton and regeneration from its remains. If Brandt's theory that nitrate is controlled by denitrifying bacteria were true to any considerable extent, this would not be so.

The nitrogen cycle, as far as it is known at present, may be summed up in a diagram (Text-fig. 15).



Text-fig. 15.

VI. SUMMARY.

1. The conditions for the production of plankton in the sea are discussed in the light of present-day knowledge.

2. An account is given of a preliminary investigation of the metabolism of cultures of marine plankton diatoms. The consumption of nutrient salts proved somewhat variable. A rapid increase in nitrite was observed whenever a culture began to die off.

3. The distribution of nitrate and nitrite in the area covered by the Expedition is described, with special reference to the upper 300 metres of the water column. The figures are discussed in detail in conjunction with oxygen, stability, transparency and phytoplankton data, especially in their bearing on the production of plankton in the area.

4. Our knowledge of the nitrogen cycle in the sea is reviewed and discussed in the light of recent bacteriological and other investigations.

APPENDIX: ANALYTICAL METHODS.

I. Diatom Counts.

The following methods were used in estimating the diatom population of experimental cultures:

(*a*) When the culture was young, and diatoms relatively few, 11 ml. were measured into a tapered centrifuge tube, and centrifuged for 10–15 minutes. About 10 ml. were then pipetted out, and the remaining 1 ml. centrifuged again for a further 5 minutes. The supernatant fluid was then carefully removed with a fine pipette until 0·1–0·2 ml. remained. This was stirred and transferred with the pipette to a slide, and the whole counted under a $\frac{2}{3}$-in. objective. The losses in this procedure have been shown by Gran (1929*a*) to be about 10%, and the 11 ml. are therefore calculated as 10.

(*b*) As the cultures became denser, the numbers to be counted by this method became inconveniently large, over 4000 cells per sample being counted on one or two occasions. This was extremely fatiguing and time-consuming, and some way of reducing the numbers was sought. Accordingly in a few cases 1 ml. of the culture was added to 10 ml. of filtered sea-water in a centrifuge tube, and treated as above.

(*c*) In many cases, where the population had risen to 1000 or more diatoms per ml., the simpler procedure was adopted of measuring 0·1 ml. direct on to the counting slide with a graduated pipette. Such counts were always made in duplicate, and when time allowed, in triplicate.

(*d*) In experiments with *Nitzschia closterium*, where the cells are exceedingly small and the numbers large, counting was done with a Thoma hæmacytometer slide (kindly lent me by Mr. H. W. Harvey), in which the volume over the ruled area was 0·1 c.mm. Such counts were usually made in triplicate, more being done in some cases, so that at least 400 cells were counted from each sample.

Individual counts did not usually differ by more than 15%, and the errors as a whole are believed not to exceed 10%.

II. Nitrate.

Nitrates were determined by the Denigès-Harvey method, using reduced strychnine in sulphuric acid (Harvey, 1928 ; Cooper, 1932).

The reagent for the John Murray Expedition was made up in July, 1933, at the Plymouth laboratory according to the directions given by Harvey. In all, four reductions were carried out, each of 10 gm. of strychnine sulphate, 750 gm. of zinc, and about 1 litre of hydrochloric acid added in portions of 100 ml. or less at intervals of 3–4 hours. The reaction flask was heated meanwhile in a boiling water bath on an electric hot-plate. Of these the first was found to have too large a blank value, and was rejected. The others were mixed in portions with 10 times their volume of " nitrogen-free " sulphuric acid, and decanted into the original acid bottles (about 700 ml.) when the zinc sulphate had been allowed to settle.

The acid obtained for the purpose from Messrs. British Drug Houses Limited proved to contain a certain amount of nitrate, so that the finished reagent had a considerable blank value. I am much indebted to Dr. W. R. G. Atkins for lending me a quantity of an old batch of better acid, thus enabling me to prepare a small quantity of reagent with a very small blank, for use where a low nitrate concentration was expected. I also received much help and advice in the preparation from Dr. Cooper.

It was found that the blank value of the resulting reagent is greatly affected by the time which elapses between the completion of the reduction process and the addition of the sulphuric acid. Provided the addition is made immediately the reduction is complete, it is unnecessary to take great precautions to exclude nitric fumes during the reduction process, since any nitrate which finds its way into the mixture is immediately reduced by the nascent hydrogen. This is believed to be the reason for the appearance and subsequent rapid disappearance of the " pink flush " described by Harvey at each addition of hydrochloric acid to the reduction flask.

In use, 5 ml. of reagent was added to 4 ml. of each sample in small stoppered bottles, which were then stored on their sides in the dark. It was thus found possible to pour the clear liquid into the colorimeter cup without disturbing the slight precipitate of calcium sulphate which nearly always forms. Eighteen to twenty-six hours later the colour was measured in arbitrary units by comparison with a standard solution of safranin in a Leitz Duboscq colorimeter.

At least once during the use at sea of each bottle of the reagent, a calibration curve was constructed by determining the colour developed by known amounts of nitrate added to surface sea-water containing very little or none. Plotting " colour units " against nitrate content then normally gives a linear relation up to 300 or 400 mg. nitrate–N/m^3. From the slope of the graph so obtained a factor was derived, which was used to convert " colour units " into nitrate content after subtraction of a blank correction. Where the linear relation did not hold, values were read off direct from the curves. Both factor and blank correction were found to vary considerably between different batches of reagent, but between bottles of the same batch the differences were slight.*

As the distilled water prepared on board always seemed to contain considerable

* Sund (1929) investigated the temperature characteristics of the colour reaction, and found that the colour developed is affected by the temperature. In the ship's laboratory temperature was nearly constant, and as the samples and standards experienced similar conditions, no correction has been applied.

quantities of nitrate, some difficulty was experienced in determining the blank correction. However, as each batch of reagent was found to give fairly constant low figures for surface and 10-metre samples at a number of different stations, this minimum value was taken to indicate complete absence of nitrate, and was applied as a blank correction to the other readings. There is therefore the possibility that the absolute amounts of nitrate recorded are slightly too low throughout, but this does not affect the relative accuracy of results in the depth series.

As fatigue was found at times to reduce the accuracy of the eye in the rather cramped conditions of the ship's laboratory, errors somewhat exceeding 5% must be regarded as possible.

For the culture experiments a fresh supply of the reagent was prepared in Cambridge in November, 1934, and proved extremely satisfactory. The procedure was varied in that 5 ml. of reagent were added to 5 ml. (instead of 4) of the sample. This was done in 1-in. Monax test-tubes, which were then covered with glass stoppers to exclude dust. Colorimetry was done with the photo-electric colorimeter described below, so that no decantation of samples was necessary.

III. Nitrite.

The standard Griess-Ilosvay method was used (Orr, 1926; Wattenberg, 1931), following the procedure described by Cooper (1933*a*), 4 ml. of the mixed reagent being added to about 100 ml. of the sample in the ordinary salinity sample bottles, of which a number were reserved for this purpose. The colour comparisons were made after 24 to 36 hours in a Sund colorimeter (Sund, 1931), using a solution of safranin in the wedge, and a pale blue glass in front of the lamp. A set of standards in surface sea-water was prepared at the same time as the samples from each station. Variations in the nitrite value of the wedge were small. With the 25-cm. colorimeter tubes used 0·1 mg. NO_2–N/m^3 was easily detectable. By this method no blank correction is required except in calculating the nitrite value of the wedge, where it was applied when necessitated by the presence of traces of nitrite in the water used for the standards.

For the analyses in connection with the culture experiments the photo-electric colorimeter was also used. Since this instrument only requires 25-ml. samples, the reaction was carried out in 1-in. Monax test-tubes. At first the samples were left overnight for the colour to develop, but they were then found to absorb appreciable amounts of nitrite from the air of the laboratory. This was overcome by leaving them only two hours, which was found quite sufficient.

IV. Phosphate.

Estimations of phosphate in the culture experiments were made by the Denigès-Atkins method, with Kalle's (1934) modification of using only half the amount of molybdate reagent originally recommended by Atkins (1923, etc.). Comparison was made in the photo-electric colorimeter from 10 to 20 minutes after adding the reducing agent.

V. Ammonia.

It was intended to carry out routine ammonia determinations during the John Murray Expedition by the direct nesslerization method introduced by Wattenberg (1928)

(see also Cooper, 1933*a*). Preliminary experiments at Plymouth showed that this method is not without difficulties, however, and all attempts to use it at sea failed completely. The Witting-Buch method involving preliminary precipitation of those constituents of sea-water which interfere with the Nessler reagent (Witting, 1914; Robinson and Wirth, 1934*b*) was then tried, and promised to work well. But it was found that the time and laboratory space required were altogether excessive, and determinations (of rather doubtful value) were only made at two stations.

VI. Phytoplankton Measurements.

The water meter with its wooden jacket and the brass bucket for the bottom of the net were made by Messrs. Casella & Co. to Mr. Harvey's design, and did not differ essentially from those since described by him (Harvey, 1934*a*). The canvas mouth and the silk nets were supplied by the Plymouth laboratory.

In use the net was weighted below, so that although lowered with mouth open, it did not fish on the downward journey, which was made with the brake held on by an elastic cord. When it had reached the bottom of the column of water it was intended to fish, the winch was stopped, and the first messenger sent down the wire. This released the brake, and hauling was at once started, a stop-watch being started at the same time. A little before reaching the top of the desired column the second messenger was sent, and the winch then stopped until its arrival, at which moment the watch was stopped. The net being now throttled, and the brake reapplied to the meter, the apparatus was hauled to the surface and got back on deck. From the reading of the meter and the time taken for the haul the volume of water which had passed through the net could be calculated according to the calibration of the meter made by the National Physical Laboratory. A standard portion of the contents of the bucket was poured off after vigorous stirring into a jar and preserved with formalin. The rest was strained through a disc of bolting silk, and the plant pigments dissolved out in 80% acetone as described by Harvey. The standards used for the colour comparison were made according to his original formula as follows:

Potassium chromate 2%	6 ml.
Chromic sulphate 1%	40 ml.
Sulphuric acid	a drop.

A solution containing 0·46 ml. of the above in 50 ml. contains 1 " colour unit " per ml.

After the return of the expedition the set of standards used was compared with Harvey's original set and found to agree exactly. The results here recorded are therefore directly comparable with his figures for the English Channel (Harvey, 1934*b*).

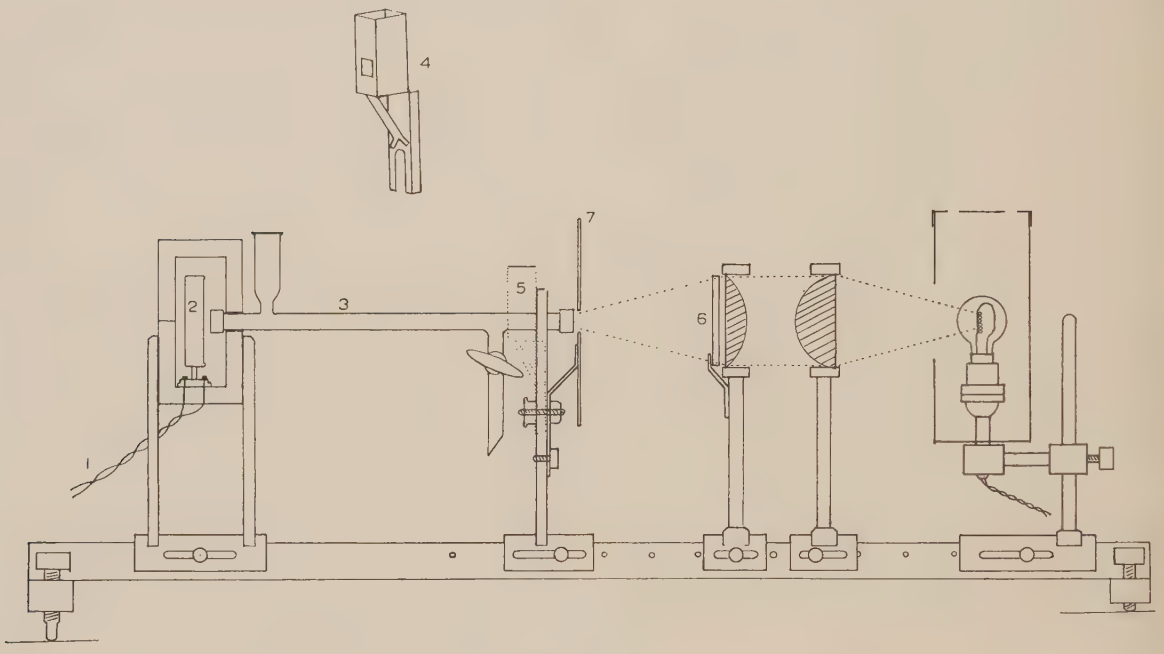
VII. A Photo-electric Colorimeter for Use with Faintly Coloured Solutions.

This apparatus was designed for the measurement of the very faint colours often met with in oceanographical chemistry, in order to increase both accuracy and speed of working, and at the same time reduce the considerable fatigue occasioned by a long series of optical comparisons.

It is based (see Text-fig. 16) on an optical bench made of a length of ordinary " angle

brass " mounted at the ends on cross-pieces of square brass rod, each of which carries two levelling screws. The bench carries a simple adjustable lamp-house, a condensing system of two lenses, the mounting of one of which also carries a light-filter, a fitting to carry one end of the glass colorimeter tube, and a light-tight wooden box, which carries the other end of the tube and contains a Weston " Photronic " photo-electric cell mounted in a radio valve-holder. A series of tapped holes along the bench and slots in the bases of all the fittings enable these to be clamped in any desired position by set-screws with knurled heads. The whole may be easily constructed in any reasonably well-equipped workshop.

The glass colorimeter tube is about 1 cm. bore, and has a tubulure 2 cm. from one end, and an outlet tube provided with a tap about the same distance from the other end ;



Text-fig. 16.

it is painted black outside to keep out extraneous light. Any desired length of tube could be used, but 25 cm. has been found satisfactory for the depths of colour commonly met with in the analysis of phosphates and nitrites in sea-water. The ends of the tube are ground off square and smooth, and are closed by optically worked glass discs 15 mm. in diameter, such as Messrs. Leitz supply for the bottoms of their colorimeter cups. The discs are held in place by brass caps, which screw on to brass sleeves cemented to the tube with plaster-of-paris. To give the plaster a key on the glass, the outside of the tube is slightly roughened by grinding on a carborundum wheel for about a centimetre from the end. Shallow grooves are turned in the inside of the sleeve for the same purpose. If the end of the glass tube is carefully smeared with a very little tap grease before the disc is put on, there should be no difficulty in making a tight joint.

For use with strongly coloured solutions, such as are given by the Denigès-Harvey reagent for nitrate estimation, a fitting was made of thin sheet brass (see inset in the figure) to carry a 1-in. diameter test-tube in a vertical position. This clamps on to the

tube support when the tube is removed. Two square windows allow a beam of light to pass horizontally across the test-tube and into the box housing the photo-electric cell, which is brought up close. It was found a simple matter to select test-tubes which did not differ enough in bore to cause appreciable errors. The colour reaction is carried out in these tubes, which are then put into the apparatus in turn, thus avoiding all pouring of the acid solution from one vessel to another.

For light source an ordinary 12 v. 36 w. gas-filled motor-car headlamp bulb supplied through a transformer from the A.C. mains was found very satisfactory. The addition of a rheostat and an ammeter makes it possible to obtain constant illumination from day to day, and is desirable but not essential.

The photo-electric current may be measured by any suitably sensitive galvanometer of low resistance. The one used hitherto is a small one with a resistance of about 50 ohms and a sensitivity of 2μA per scale division. Being intended for use as a null-point instrument, it has too short a scale, and is not ideal. One of the Cambridge Instrument Company's " Unipivot " instruments of similar sensitivity should be very satisfactory, and if suitably mounted, would allow the apparatus to be used on board ship.*

Provided it is approximately complementary to the colour of the solution to be measured, the exact transmission of the light-filter used is immaterial. For phosphate determinations a " signal red " made by the Corning Glass Company has been used ; for nitrate and nitrite a " signal green " made by Messrs. Chance Brothers.

The procedure in making a set of determinations is as follows : The appropriate filter is inserted, the tube filled with distilled water, and the light turned on some ten minutes before it is required to begin measurements. This allows the lamp filament to reach a constant temperature, and avoids a slight fatigue effect exhibited by the photo-electric cell in the first few minutes. The illumination is then adjusted so as to give exactly a full-scale deflection on the galvanometer, this being read as zero. The tube is then emptied, and the standards and samples poured in in turn, allowing a few seconds for drainage between each, and the reading taken in each case as soon as a steady value is attained. Each reading is thus the difference in galvanometer scale units between the deflection given by the sample and that given by distilled water.† At the end of a series the tube is again filled with distilled water, and the reading taken to make sure there has been no change in illumination.

Some care is needed to avoid the inclusion of bubbles when filling the tube. When the slope of the apparatus is adjusted to the minimum which gives effective drainage, it is found that after some practice a bubble is very rarely trapped in filling. When one is caught, its presence is always revealed by an unsteady or abnormally high reading. In changing from distilled water to sea-water samples, and *vice versa*, it is always necessary to rinse the tube carefully, or the turbulence caused by a mixture in the tube gives rise to considerable errors.

A fresh set of standards is prepared as usual for each set of analyses. The readings given by these normally lie on a smooth curve, when plotted against the concentration, and any faulty standard can thus be detected and ignored. Phosphate calibration curves hardly vary from day to day, and no blank correction should be necessary. In nitrite

* Dr. W. R. G. Atkins disputes this statement.

† The fact that the illumination-current relation of the photo-electric cell is not linear is immaterial, since a direct calibration against known standards has to be made in any case.

analyses the curves are more variable, and blank corrections may have to be applied. In this case the blank may be due either to nitrite present in the distilled water, or to absorption of light by the reagent itself. In the first case the amount present is added to the values given to the standards, and the curve plotted accordingly ; in the second case the same absorption is present in the samples, and no correction is therefore necessary.* The two can be distinguished by preparing a second " zero " standard (distilled water), to which the reagent is added immediately before comparison, thus allowing no time for colour development. The difference between this and the " zero " standard prepared with the others gives the true blank due to nitrite in the distilled water. This is usually small or absent, while the " false blank " may be considerable, especially if the reagents are not very fresh.

In nitrate estimations the constancy of the colorimetric conditions may be checked each day on a set of permanent safranin standards, and the variations in the calibration curve so obtained should be extremely small. From this curve the samples for analysis can be read off in " colour units ", and the constants required for converting these into nitrate concentration must be determined separately for each batch of reagent (Cooper, 1932).

Owing to small fluctuations in the mains voltage, and to the fact that the galvanometer used was not ideal, the accuracy so far attained does not exceed that of visual methods under good conditions, and is indeed not as great as that claimed by Kalle (1934) using the Pulfrich photometer. However, as he has shown that the errors inherent in the method as a whole exceed the colorimeter error, there is nothing to be gained from extreme accuracy in the colorimetry. The same is true of many colorimetric methods. The errors in the analyses so far carried out with the apparatus are approximately as follows : Nitrite $\pm 0{\cdot}1$ mg./m^3 for concentrations up to 10 mg. N/m^3 ; phosphate $\pm$ 2–3% ; nitrate $\pm$ 5%.

The great advantage of the instrument is the enormous saving in time,† and especially in fatigue. It was observed that nitrate estimations could be carried out in approximately one-fifth of the time required with a Duboscq colorimeter. In this case the accuracy is probably also greater where a great number of comparisons have to be made, owing to the eye's loss of accuracy from fatigue. A considerable gain in accuracy is also to be anticipated in the case of bad colorimetric colours such as the yellow of Diénert and Wandenbulcke's method for silicates. The instrument has, however, the defect that errors are easily introduced by any turbidity in the solution. Thus in work on diatom cultures it was found essential to filter the samples.

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* This effect gives rise to no blank value, when comparison is made by eye, since the " zero " standard and not distilled water is used as the basis for comparison.

† Dr. W. R. G. Atkins points out that there is no saving in time as compared with Helmer tubes. While this is probably so, most workers would find a considerable gain in accuracy.

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